AN ANALYSIS BY SELECTION AND CROSSING, OF GENETIC FACTORS INVOLVED IN DEFECTIVE VENATION, A VARIABLE CHARACTER OF THE PARASITIC WASP, *HABROBRACON JUGLANDIS* (ASHMEAD)

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INTRODUCTION

This insect was first used for genetic research by P. W. WHITING in 1919, and it has been bred extensively in his laboratory since that time. A large amount of data has been gathered dealing with the behavior of several mutant genes, chief among which are orange eye and defective venation (WHITING 1921 a, b, 1924). Several stocks of the wasp show a vein defect consisting of partial or complete absence of the fourth branch of the radius. The origin of this defect and its variations are described by WHITING (1924). The progeny of a female possessing a factor for this defect range

from normal to complete absence of vein. The amount of defect in any individual is estimated and placed in one of nine arbitrary grades. In the tabulations 0 signifies the normal type, or no defect. The number 1 represents the absence of one-fourth of the vein in one wing, 2 the absence of one-half of it in one wing or one-fourth of it in both wings, and so on, 8 meaning the complete absence of both veins. The defect is not symmetrically disposed. All the progeny of a mating, or series of matings, are graded and the mean is taken. This mean may be that of the total number of wasps, including type individuals (Mn); or it may be that of the defectives only (Md). The percentage of defectives (percent d) is usually added. It is found that at a given temperature the Mn, Md and percent d are fairly constant within a pure stock. The main portion of the present experiments has to do with an investigation of the behavior of these constants under selection and crossing.

STOCK 3

This stock, called the Orange High-defective stock, was isolated in December, 1920, by WHITING. During the year 1901 it gave the following averages (WHITING 1924, p. 28):

	Percent d	*Md*
Males	95.2	6.61
Females	85.8	5.84

Cultures made in 1922 averaged higher:

	Percent d	*Md*
Males	95.8	6.97
Females	92.7	6.39

WHITING thinks that "it is probable, therefore, that a change in the direction of increased defectiveness has occurred without conscious selection." The early cultures of the present experiment gave (839 wasps):

	Percent d	*Md*
Males	99.4	6.47
Females	95.1	5.98

The percentages are higher than in the previous year, but the means of the defectives are lower. Possibly this discrepancy is due to a difference in estimating extremely low-defective specimens. If the investigator is inclined to call an insect with only a faint indication of defect a type specimen, the percentage will be lowered and the average raised. WHITING (1922, table J) gives only 0.22 percent for males of grade 1, while the wasps bred in 1923 show 1.19 percent defective males in this grade. Further allowance must be made for differences in cultural conditions when

experiments are conducted under different circumstances. In a word, the above data, while suggestive, are not sufficiently critical to demonstrate that there has been a change in the stock since 1922.

Selection within stock 3

The purpose of the first of the present series of experiments was to determine whether a change in the average or in the percentage of defectives can be induced by selection. Accordingly, two lines of cultures were conducted under identical conditions of temperature and food supply; in line A matings were made between wasps of low defect, whenever possible from type specimens; in line B the selection was for high defect, and this meant that in nearly all cases crosses were made between two completely defective individuals (grade 8). Line A was carried through nineteen generations; line B through twenty-two generations. These cultures were begun in the fall of 1922 at exactly 30° C; but it was found that at this temperature the number of low-grade defectives in line A was so small that it was difficult to get enough of them to make reasonable progress; for this reason the temperature was lowered two degrees the following spring and kept there, with some unintentional minor variations, until December, 1923, after which it was again raised to 30 degrees. This change of temperature complicates the interpretation of the data, since it is necessary to compare the wasps of the two lines that were bred simultaneously, rather than those of the comparable successive generations in each line. The tables and graphs show what effect the two-degree drop had on all wasps.

Data

Table 1 summarizes the nineteen generations of cultures in line A. The numbers of the first six generations were not large enough to justify separate summaries, and they have been grouped in such a way that group 2 comprises the second and third generations, and group 3 the fourth, fifth, and sixth generations. Groups 4 to 16 correspond to generations 7 to 19. Table 2 summarizes the wasps of line B that were bred simultaneously with each of the sixteen groups in table 1, irrespective of the generations they represent.

A study of these tables, as well as an examination of the data of previous work (WHITING 1924) shows that the number of males obtained is generally in excess of the number of females; also, that the average of defect is higher in the males than in the females. The first of these two facts is accounted for when we remember that males are produced from unfertilized eggs; if the male of any particular mating happens to be sterile, or

TABLE 1

Showing that continued breeding from stock-3 wasps selected for low defect produced no significant change in the average of defect or percentage of defectives.

GROUP	SEX	GRADE OF DEFECT									TOTAL	MEAN DEFECT	PERCENT DEFECTIVE
		0	1	2	3	4	5	6	7	8			
1.....	♂♂	1	2	3	0	11	6	15	18	22	78	6.07±.15	98.72
	♀♀	7	2	3	2	9	8	12	12	11	66	5.05±.21	89.40
2.....	♂♂	1	1	3	4	6	5	15	17	40	92	6.50±.13	98.92
	♀♀	1	2	6	3	21	7	25	27	32	124	5.94±.11	99.20
3.....	♂♂	3	1	4	2	16	7	10	14	35	92	6.00±.15	96.74
	♀♀	7	1	7	8	20	4	14	16	23	100	5.19±.14	93.00
4.....	♂♂	9	9	10	13	39	26	51	55	65	277	5.65±.08	96.75
	♀♀	13	10	11	9	33	34	46	46	23	225	5.08±.10	94.89
5.....	♂♂	1	1	3	2	5	3	12	10	19	56	6.10±.18	98.20
	♀♀	6	6	3	6	8	6	9	8	8	60	4.43±.23	90.00
6.....	♂♂	14	14	12	10	31	17	39	43	54	234	5.27±.11	94.00
	♀♀	2	0	1	4	2	4	0	7	3	23	5.00±.34	91.30
7.....	♂♂	1	3	0	3	9	7	14	19	13	69	5.85±.15	98.55
	♀♀	1	1	3	1	4	14	29	23	10	86	5.94±.11	98.84
8.....	♂♂	27	17	11	18	42	36	70	72	56	349	5.19±.09	92.27
	♀♀	13	3	12	11	25	37	55	36	17	209	5.08±.10	93.78
9.....	♂♂	13	9	15	20	49	39	78	93	36	352	5.39±.07	96.31
	♀♀	9	2	3	8	11	8	34	18	4	97	4.92±.15	98.72
10.....	♂♂	10	2	6	8	19	16	50	50	17	178	5.50±.10	94.39
	♀♀	10	3	5	7	18	19	51	20	11	144	5.10±.12	93.00
11.....	♂♂	26	15	29	20	43	32	75	47	27	314	4.65±.09	91.72
	♀♀	15	3	10	18	31	13	66	37	9	202	4.91±.09	92.58
12.....	♂♂	23	6	11	9	17	20	60	49	38	233	5.28±.12	90.13
	♀♀	1	2	4	2	8	8	32	13	12	82	5.70±.11	98.78
13.....	♂♂	3	2	7	4	14	9	56	54	60	209	6.33±.08	98.75
	♀♀	8	3	6	6	13	16	59	37	26	174	5.67±.10	95.41
14.....	♂♂	3	5	4	3	7	9	36	55	55	177	6.41±.09	98.30
	♀♀	4	1	5	4	8	14	60	36	19	151	5.91±.09	97.36

TABLE 1 (continued)

GROUP	SEX	GRADE OF DEFECT									TOTAL	MEAN DEFECT	PERCENT DEFECTIVE
		0	1	2	3	4	5	6	7	8			
15.....	♂♂	1	1	2	0	4	3	12	13	26	87	6.87±.11	98.85
	♀♀	5	0	7	0	2	2	10	10	5	41	5.00±.28	87.80
16.....	♂♂	1	2	5	5	5	4	15	13	15	65	5.66±.18	98.47
	♀♀	2	2	3	2	4	5	17	6	5	46	5.20±.21	95.66
All grades	♂♂	137	90	125	121	317	239	608	647	578	2862	5.58±.03	95.28
	♀♀	104	41	89	91	217	199	519	352	218	1830	5.28±.03	94.27

TABLE 2

Showing that continued breeding from stock-3 wasps selected for high defect produced no significant change in mean defect or percentage of defectives.

GROUP	SEX	GRADE OF DEFECT									TOTAL	MEAN DEFECT	PERCENT DEFECTIVES
		0	1	2	3	4	5	6	7	8			
1	♂♂	2	1	1	1	4	2	16	22	32	81	6.64±.12	97.53
	♀♀	4	0	7	2	6	11	13	20	21	84	5.77±.16	95.12
2	♂♂	3	1	2	4	21	12	37	61	120	261	6.75±.07	98.86
	♀♀	10	6	7	7	16	15	36	54	57	208	5.91±.11	95.20
3	♂♂	1	0	1	1	3	2	13	15	31	67	6.86±.13	98.51
	♀♀	5	1	5	2	15	3	16	22	28	97	5.83±.16	94.85
4	♂♂	8	9	9	13	38	31	75	83	113	379	6.09±.02	97.89
	♀♀	4	3	8	7	26	13	60	54	54	229	6.02±.08	98.26
5	♂♂	0	0	6	2	19	9	26	27	31	120	6.10±.11	100.00
	♀♀	4	3	3	5	17	16	16	16	9	89	5.08±.15	95.50
6	♂♂	6	5	6	8	6	7	24	31	29	122	5.63±.15	95.08
	♀♀	6	1	2	2	5	6	8	4	4	38	4.44±.28	84.21
7	♂♂	4	9	11	9	29	29	53	80	63	287	5.95±.08	98.61
	♀♀	12	3	6	7	7	17	38	39	17	146	5.38±.13	91.78
8	♂♂	10	3	13	15	43	33	52	42	19	230	5.22±.09	96.07
	♀♀	10	2	15	17	20	30	42	39	8	183	4.93±.10	94.54
9	♂♂	13	9	30	28	53	63	160	93	42	491	5.35±.06	97.36
	♀♀	11	3	13	15	37	34	110	74	42	339	5.62±.07	96.76
10	♂♂	2	3	10	5	27	15	40	46	28	176	5.72±.09	98.87
	♀♀	10	6	12	10	28	29	73	42	16	226	5.20±.09	95.58

TABLE 2 (continued)

GROUP	SEX	GRADE OF DEFECT									TOTAL	MEAN DEFECT	PERCENT DEFECTIVE
		0	1	2	3	4	5	6	7	8			
11	♂♂	8	7	18	14	29	37	95	59	23	290	5.35±.08	97.24
	♀♀	14	9	16	15	36	37	82	40	22	271	5.03±.09	94.84
12	♂♂	11	7	15	12	26	21	69	100	72	333	5.92±.07	96.70
	♀♀	5	2	5	5	12	18	43	44	37	171	6.00±.16	97.08
13	♂♂	8	4	15	6	12	20	47	88	78	278	6.19±.08	97.12
	♀♀	8	3	11	9	16	30	81	76	42	276	5.87±.08	97.10
14	♂♂	7	5	9	10	17	24	81	151	171	475	6.63±.05	98.53
	♀♀	5	3	6	7	7	28	97	87	48	288	6.17±.06	98.27
15	♂♂	5	1	5	7	9	15	48	84	98	272	6.62±.07	98.16
	♀♀	12	9	7	10	10	15	58	62	28	211	5.56±.10	94.30
16	♂♂	4	0	3	7	4	6	17	44	20	105	6.12±.13	96.19
	♀♀	6	3	5	7	13	10	42	35	20	141	5.62±.12	95.75
Odd culture	♂♂	6	2	4	0	9	3	18	40	95	145		
	♀♀	10	6	7	6	17	12	24	25	30	137		
All grades	♂♂	98	66	158	142	348	329	871	1066	1045	4122	6.03±.02	97.47
	♀♀	136	63	135	133	288	324	839	733	483	3134	5.59±.03	95.66
												5.81	96.57

if the mating for any reason is unsuccessful, the offspring will be all males. The second fact, the higher average defect of the males, is probably correlated with their haploid character, inasmuch as in females the normal allelomorph for some of the defect factors may be present. These two facts taken together make it necessary, if successive groups are to be compared, to compute the average of each sex separately; otherwise those cultures in which the males predominate will show a higher average than those in which the sexes are approximately equal.

Diagram 1 shows the distribution of the amount of defect. The mode for females is at grade 6, for males at 7. The minor modes at grades 2 and 4 may be due to causes operative in development (POTTER, unpublished), but their significance is not clear. The females show a smaller percentage of wasps in the high grades and a larger percentage in the low grades than the males. The distribution for lines A and B is very similar.

Diagram 2 shows how the sixteen groups of line A compare with those of line B as to average of defect and percentage of defectives. The mean of

both lines dropped when the temperature was lowered, and minor fluctuations are, in the main, parallel. Line A is, in general, lower than line B; the exceptions in percentages are probably not significant, and the lower grades of defect of line B in groups 6, 7, and 8 may possibly be accounted for by the small number of females or accidental excess of type individuals. The conspicuous ups and downs are doubtless due to accidental lapses in incubator efficiency or to changes in food supply. The significant thing is that the two lines rise and fall together, indicating that the variations are environmental rather than genetic.

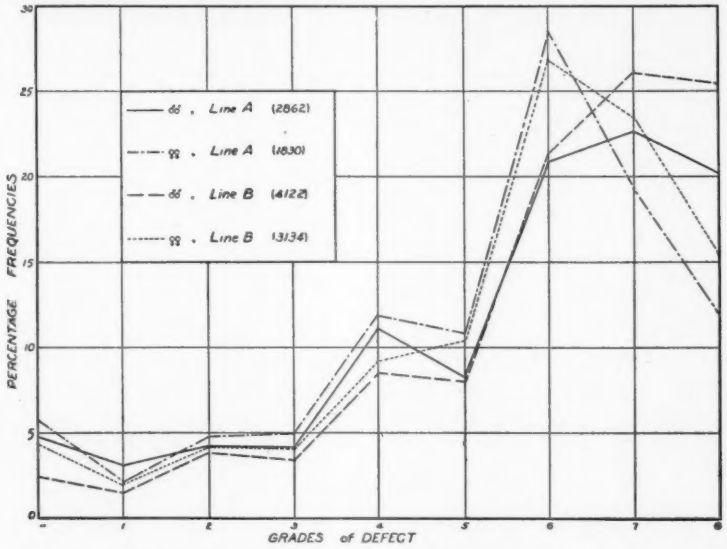


DIAGRAM 1.—The percentage of wasps (ordinates) in each grade of defect (abscissae) in four lines of stock 3. Line A was selected for low defect, line B for high defect.

At times the progenies of individual cultures through two or three generations gave promise of yielding to selection. The best example of this was No. 836 in the fourteenth generation of line B. It maintained an Mn of 6.40 and a percent d of 98.75 through four generations; but the later offspring fell lower, and the mean of about a thousand descendants of this culture was 6.25, the last ones being still lower.

Conclusions

A careful study of the data of this experiment justifies the conclusion that selection through twenty-two generations has had no effect on stock 3,

for the difference between lines A and B is not increased as selection proceeds. The probable error indicates that the difference between any two of the groups is not significant; however, one cannot disregard the persistence of this difference in thirteen of the sixteen groups; moreover, the difference between the totals of the two lines for the Mn is $.45 \pm .034$ percent for males, and $.31 \pm .042$ percent for females. This difference is evidently significant and an explanation is necessary. It is possible that the first few generations did show the effect of selection through the segregation of a modifying factor, or a mutation may have occurred in one of the lines. The writer suspects, however, that the difference may be due,

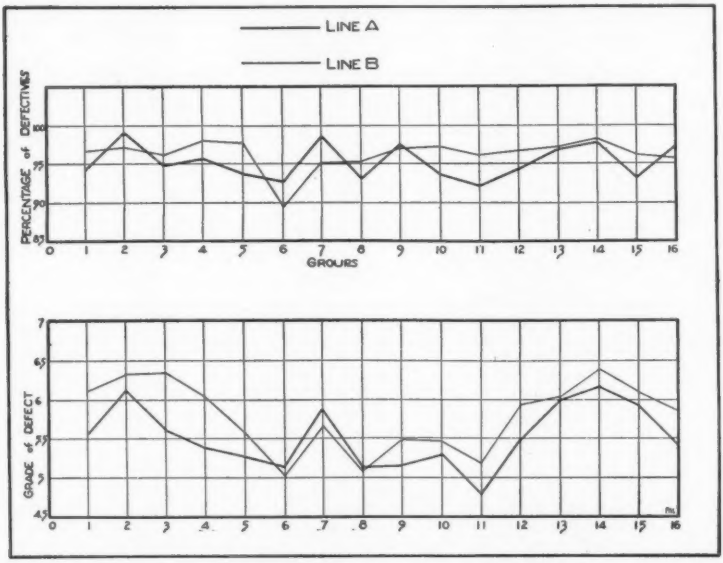


DIAGRAM 2.—Percentage of defectives and of mean defect (ordinates) in the successive groups (abscissae) of lines A and B, stock 3.

in part at least, to an entirely different cause. When examining the wasps it is often difficult to decide whether to put an individual in, say, group 5 or group 6. The decision must be made without accurate measurement, and subconsciously one prefers 5 to 6 in line A and *vice versa* in line B. Such inaccuracy, even if only occasional, may affect the results preceptibly. At any rate, in view of such a possibility, it is thought safer not to attach too much significance to the constant difference between lines A and B.

STOCK 14

When stock 14 was first bred from in October, 1923, there was no intention of performing a selection experiment. The stock, which is black-eyed and of mixed origin, was known to contain one or more factors for defect different from that of stock 3; and it was thought that by proper crossing and selection from the cross, a strain might be obtained containing both sets of factors. In keeping up this stock, only individuals of high defect were bred, and it soon became evident that a change of considerable magnitude was taking place. The selection was not rigid, no attempt being made to control the matings; a high-defective female was selected for each new culture, but as a rule no attention was paid to the male

TABLE 3

Showing the effect produced by selective breeding in stock 14.

GROUP	SEX	GRADE OF DEFECT									TOTAL	MEAN DEFECT	PERCENT DEFECTIVE
		0	1	2	3	4	5	6	7	8			
1 and 2...	♂♂	60	20	25	9	18	10	9	1	4	156	2.00±.12	61.54
	♀♀	58	19	10	14	16	3	4	3	0	127	1.61±.12	54.38
3.....	♂♂	90	42	42	22	28	8	10	5	1	248	1.80±.08	63.72
	♀♀	59	20	33	22	24	10	16	4	0	188	2.24±.09	68.62
4.....	♂♂	89	16	26	23	23	12	22	12	2	225	2.32±.07	60.45
	♀♀	53	8	8	19	15	7	15	2	0	127	2.20±.13	58.27
5.....	♂♂	67	17	29	19	43	23	48	19	3	268	3.22±.10	75.00
	♀♀	10	2	5	8	21	9	13	4	0	72	3.76±.16	86.12
6.....	♂♂	118	24	54	36	44	26	64	35	15	416	3.11±.09	71.64
	♀♀	51	14	31	20	32	20	35	12	4	219	3.10±.11	76.72
7.....	♂♂	38	4	29	18	30	21	60	40	28	268	4.50±.11	85.82
	♀♀	21	7	18	17	21	20	35	13	1	153	3.83±.12	86.28
8.....	♂♂	30	8	18	13	14	14	33	23	29	182	4.40±.14	83.52
	♀♀	25	6	19	17	15	13	19	13	3	130	3.45±.14	80.77
9.....	♂♂	22	12	13	12	13	9	31	24	19	155	4.37±.15	85.81
	♀♀	35	9	14	8	15	18	22	12	5	138	3.38±.17	74.64
10.....	♂♂	3	1	4	0	1	2	5	5	12	33	5.57±.32	90.91
	♀♀	5	1	0	1	1	4	1	2	9	24	5.00±.43	79.17
No. 262	♂♂	16	9	11	9	7	10	38	47	38	185	5.39±.13	91.35
	♀♀	14	8	10	12	11	18	22	21	15	131	4.55±.15	89.34

involved. After ten generations of this kind of selective breeding the percentage of defectives had risen from 57.94 ± 1.9 to 85.04 ± 2.7, and the average of defect (Mn) from $1.81 \pm .12$ to $5.29 \pm .43$. One strain in particular retained through four generations a percentage of 90.35 ± 1.9 and an average defect of $4.97 \pm .15$. The results of this part of the experiment are shown in table 3 and diagrams 3 and 4.

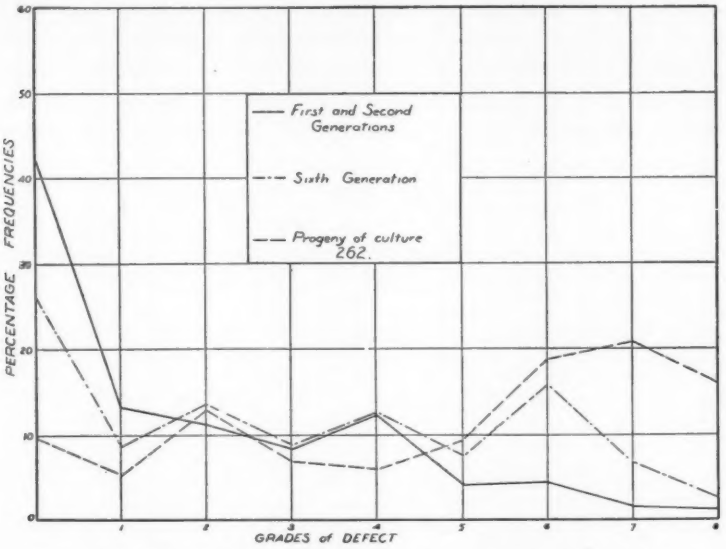


DIAGRAM 3.—Distribution of wasps with respect to grade of defect, in stock 14.

When seeking an explanation of the difference between this stock and stock 3 with reference to the results of selection, it may be observed that stock 3 was probably homozygous from the start, and that stock 14 had not yet been sufficiently inbred to eliminate heterozygosis of the various factors. It is desirable that more work be done to determine the limits beyond which selection will not be effective. If no such limit is reached, the conclusion would be justified that mutations of minor grade are occurring (ZELENY 1922).

BREEDING STOCK 3 TO STOCK 14

The method used

After twenty-two generations of selective inbreeding it was fairly well established that the defect in stock 3 is due to a single unit factor, or at

least to closely linked factors (EAST and JONES 1919). This gene is denoted by *d* and its allelomorph by *D*, though dominance is irregular (WHITING 1924, p. 12). Stock 14 evidently has one or more factors for defect that are independent of *d*; for convenience these may be denoted by *l*, *m*, *n*. In order to bring all these factors together in one individual an experiment was decided upon which consists essentially in breeding wasps with *d* derived from stock 3 to stock 14. The fact that the female reproduces parthenogenetically and that the males thus produced are haploid, is a great aid in analyzing the effects of each cross. Of course, theoretically,

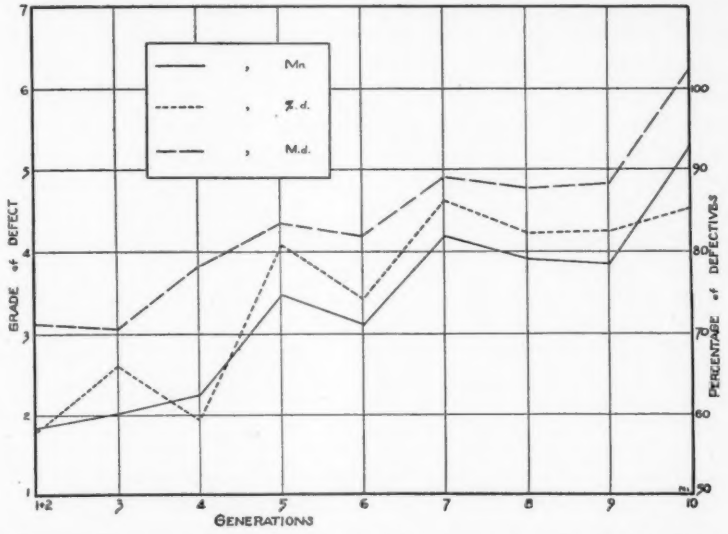
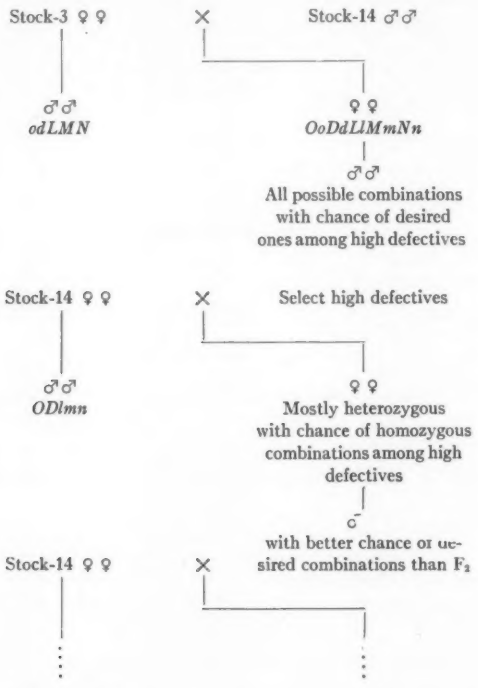


DIAGRAM 4.—Percentage of defectives and mean defect of successive generations of stock 14.

the combination desired could be obtained by haphazard selection from an ordinary F_2 generation; but since the phenotype gives no reliable clue to its genetic constitution, and since presumably several genes are involved, the chances of obtaining the right combination are very small, and many hundreds of individuals would have to be tested. For these reasons the following scheme was decided upon (WHITING 1923, 1924, p. 30). This means that the females of F_1 are bred parthenogenetically, and since the resulting males are haploid it is easy to make an analysis of the genetic constitution of the females used. From the males of F_2 a selection is made of individuals with high defect for further breeding,

this time with stock-14 females. If one uses four or five such males one may be reasonably certain that some of them possess the factor *d* and in addition one or more of the factors *l*, *m*, *n*. If the factor *d* should chance to be absent, the low averages resulting in the next generation would suffice to detect this fact. If all the factors *l*, *m*, *n*, are not present,—and the chance is that they are not,—there is hope of introducing them in the next cross with stock 14.



o, orange eye from stock 3
d, factor for high defect from stock 3
l, *m*, *n*, factors for low defect from stock 14

This scheme of upbreeding results in a sort of alternation of sexual with parthenogenetic generations; the odd numbers represent the sexually produced generations, the even numbers the offspring of virgin females. In the present experiment the process was carried through twelve generations before the final crosses were made.

Application of the method

Table 4 gives the general results of this experiment. The first cross was between females from stock 3, whose constitution was 5.18 as measured by the average Mn of the males they produced;[1] and males from stock 14, whose constitution was 1.81 as measured by their brothers. The resulting heterozygous females averaged 2.81, which number, however, should be raised if it is to be compared with the averages of the parents, because the females are regularly of lower grade than the males. There were no grade-8 individuals among these females, and those of grades 6 and 7 were not isolated in time to be used. Virgin females of grades 4 and 5 only were available for the next generation, but the offspring to be expected from these should not differ seriously from the progeny of grade-8 females (WHITING 1924, p. 40, 1a). Five of these medium-grade females were bred and produced 922 male offspring with an average defect of 3.30. From these, five high-defective orange males were selected and bred with high-defective females from stock 14. The latter stock had by this time shown the effects of selection; but a check on the defective status of the females used is available in their haploid male offspring; they carried an average defect (for males) of 2.61. The heterozygous female offspring averaged 3.52, which, though on a female basis is, nevertheless, slightly higher than that of the male averages of either parent. One of these females was grade-8 but was not obtained virgin, and five females of grade 6 and 7 were used for the fourth generation (second parthenogenetic). These females, whose sisters averaged 3.52, produced 608 males that averaged 4.49. The difference here is larger than it should be because the males are expected to be higher than the females.

This process was continued to the twelfth generation and all the essential data are included in table 4. Special considerations may be grouped under the three headings which follow.

Analyses

The stock-14 females used

These were on the average higher in each succeeding generation. The following summary of the male offspring of stock-14 females used in the

[1] There is, of course, a possibility that this average is affected by patrocliny. But if patrocliny were common enough to affect the average seriously we should expect it occasionally to affect eye color, whereas the 202 males examined from this cross were all orange. Moreover, while it has been shown that, in some crosses of black males with orange females, a large percentage of patroclinous males may be expected (ANNA R. WHITING 1925), none are produced in crosses between stock 3 and stock 11. The latter stock is closely related to stock 14 of the present experiment.

mentioned generations shows how their potential defectiveness increased:

Generation	*Average defect (Mn)*	*Percent defective*
F_3.....	2.61±.08	69.94
F_5.....	4.08±.17	82.46
F_7.....	3.62±.11	78.20
F_9.....	4.45±.14	83.98
F_{11}.....	4.21±.14	84.78

These averages are in harmony with the general upward trend of stock 14 at the time.

The heterozygous females

The females of the successive sexual generations should show the combined effect of the increasing defectiveness of stock 14, and the accumulation of the factors *l*, *m*, *n*, due to the process of breeding up.

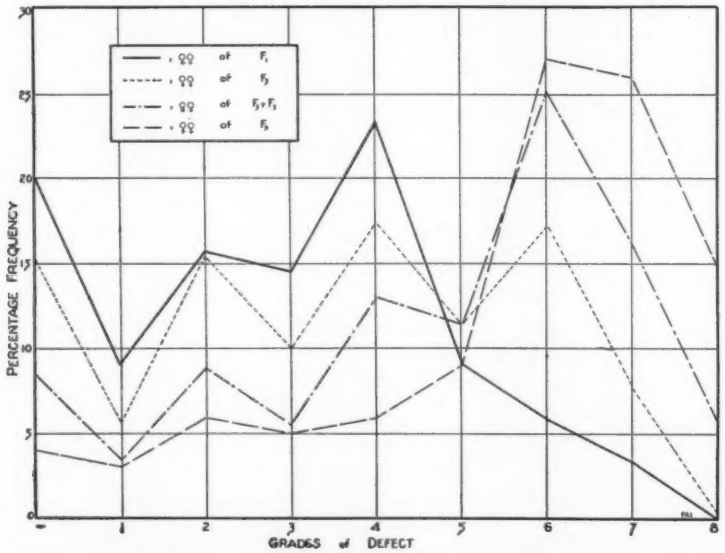


DIAGRAM 5.—Distribution with respect to grade of defect, derived from the cross, stock 14 × stock 3.

Table 4 shows the distribution of female wasps in these generations, and diagram 5 depicts it graphically (except that F_{11} is omitted). In F_1 all the females are heterozygous for all the factors involved; there is a distinct mode at 0 and another at 4; these modes suit the formula *DdLlMnNn* very well. In the successive generations the mode at 4 gradu-

TABLE 4

Stock 14 × stock 3. Summary of data brought out by means of the special method employed.

GENERATION	PARENTS	OFFSPRING: Kind	Grade of defect: 0	1	2	3	4	5	6	7	8	Total	Averages: Mean defect	Percent defective
F_1	Stock-14 ♀ ♀ ×	♂♂ (stock 3)	6	6	12	7	45	21	45	39	21	202	5.18±.10	97.03
	stock-3 ♂♂	♀ ♀ heterozygous	35	17	29	27	43	17	11	6	0	185	2.81±.10	81.08
F_2	Virgin F_1 ♀ ♀	♂♂ orange	162	26	45	22	58	25	44	36	41	459	3.01±.09	64.71
		black	130	22	44	26	58	19	53	56	55	463	3.60±.07	71.93
			292	48	89	48	116	44	97	92	96	922	3.30±.07	68.33
F_3	Stock-14 ♀ ♀	♂♂ (stock 14)	135	37	72	42	59	31	43	24	6	449	2.61±.08	69.94
	×F_2 ♂♂	♀ ♀ heterozygous	45	17	46	30	52	34	51	23	1	299	3.52±.09	84.95
F_4	Virgin F_3 ♀ ♀	♂♂ orange	84	18	26	15	19	8	44	48	63	325	4.10±.12	74.16
		black	49	7	21	15	23	8	42	43	75	283	4.87±.13	82.69
			133	25	47	30	42	16	86	91	138	608	4.49±.11	78.13
F_5	Stock-14 ♀ ♀	♂♂ (stock 14)	19	5	10	7	19	4	20	13	11	108	4.08±.17	82.40
	×F_4 ♂♂	♀ ♀ heterozygous	6	1	13	8	17	8	19	1	1	74	3.89±.13	91.90
F_6	Virgin F_5 ♀ ♀	♂♂ orange	76	7	26	12	17	2	31	31	99	301	4.53±.12	74.67
		black	63	14	22	12	16	13	28	35	97	300	4.71±.13	79.00
			139	21	48	24	33	15	59	66	196	601	4.62±.09	76.87
F_7	Stock-14 ♀ ♀	♂♂ (stock 14)	46	19	21	12	18	19	40	28	8	211	3.62±.11	78.20
	×F_6 ♂♂	♀ ♀ heterozygous	14	7	8	11	14	19	41	37	13	164	4.97±.12	91.47
F_8	Virgin F_7 ♀ ♀	♂♂ orange	89	14	32	16	22	14	42	31	144	404	4.71±.11	77.97
		black	82	16	39	10	21	11	54	42	150	425	4.90±.08	80.71
			171	30	71	26	43	25	96	73	294	829	4.81±.08	79.38
F_9	Stock-14 ♀ ♀	♂♂ (stock 14)	29	10	18	6	16	7	41	35	19	181	4.45±.14	83.98
	×F_8 ♂♂	♀ ♀ heterozygous	4	3	6	5	6	9	27	26	15	101	5.57±.23	96.00
F_{10}	Virgin F_9 ♀ ♀	♂♂ orange	29	7	10	14	12	5	11	17	63	168	4.94±.16	82.74
		black	21	2	8	5	16	7	25	20	81	185	6.16±.10	88.65
			50	9	18	19	28	12	36	37	144	353	5.54±.10	85.84
F_{11}	Stock-14 ♀ ♀	♂♂ (stock 14)	23	7	13	15	18	19	36	16	14	151	4.21±.14	84.78
	×F_{10} ♂♂	♀ ♀ heterozygous	26	4	11	9	11	11	32	21	16	141	4.39±.16	81.56
F_{12}	Virgin F_{11} ♀ ♀	♂♂ orange	23	1	7	3	2	3	7	9	28	83	4.60±.25	72.29
		black	17	3	5	2	4	2	9	6	40	88	5.20±.25	80.69
			40	4	12	5	6	5	16	15	68	171	4.90±.17	76.49

ally disappears and is replaced by a growing one at 6 (WHITING 1924, pp. 33-35). Apparently the cultures that produced F_{11} were not typical. In order to secure an adequate number of high-defective virgin females for the final crosses, ten cultures had been set; after the desired females had been obtained these cultures were discontinued. There were 141 females, and the tabulations showed that the progenies of three of the matings were abnormally low. Whatever the reason for this, the result was disastrous on tables and diagrams; F_{11} falls far below expectation.

The parthenogenetic males

The curve of the heterozygous females is quite different from that of their haploid male offspring. The distribution of the latter may be studied from table 4 and diagram 6, though the latter was designed primarily to demonstrate linkage of orange eye with low defect. Compare diagrams 1 and 3. These males are *D* or *d* with theoretically in F_2 an equal distribution of *l*, *m*, *n*, and their allelomorphs. The curve of F_2 corresponds quite well with what would be expected if the curve for males of stock 3, line B, were superimposed on that of stock 14 in its early unselected state. A comparison of the distribution of such a combination with the actual condition in F_2 is striking:

Grade	0	1	2	3	4	5	6	7	8
Expected	21.6	7.9	10.1	5.0	11.3	7.3	13.3	11.6	11.3
Observed	31.6	5.2	9.6	5.2	12.5	4.8	10.0	10.0	10.4

There are more types and fewer high-defectives than expected, but the discrepancy is accounted for by the redistribution of *l*, *m*, *n*, and their normal allelomorphs, if we assume that the latter are more effective in combination with *d* than the former. The progress in successive generations is clear-cut, as shown by the following summary:

Generation	*Average defect (Mn)*	*Percent defective*
F_2	3.30±.07	68.33
F_4	4.49±.11	78.13
F_6	4.62±.09	76.87
F_8	4.81±.08	79.38
F_{10}	5.54±.10	85.84
F_{12}	4.90±.17	76.49

The drop in the twelfth generation may be accounted for by the accidental use of wasps somatically high but with the factor *D*. Inspection of the records reveals that all the males of generation 12 were descended from one stock-14 female used as a parent in generation 11 (culture C137), and that the constitution of this female was low as measured by the

average of her sons (Mn 3.32). Compare what was said above about the heterozygous females of F_{11}.

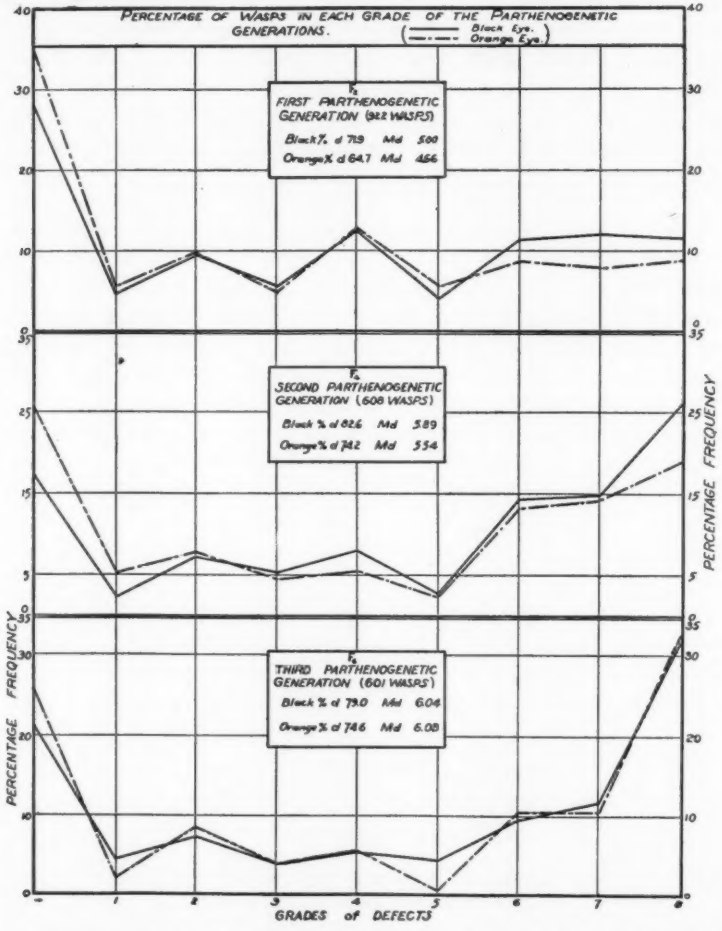

PERCENTAGE OF WASPS IN EACH GRADE OF THE PARTHENOGENETIC GENERATIONS.
Black Eye.
Orange Eye.
FIRST PARTHENOGENETIC GENERATION (922 WASPS)
Black % d 71.9 Md 5.00
Orange % d 64.7 Md 4.66
SECOND PARTHENOGENETIC GENERATION (608 WASPS)
Black % d 82.6 Md 5.89
Orange % d 74.2 Md 5.54
THIRD PARTHENOGENETIC GENERATION (601 WASPS)
Black % d 73.0 Md 6.04
Orange % d 74.6 Md 6.08
PERCENTAGE FREQUENCY
GRADES of DEFECTS

DIAGRAM 6a.—Showing the shift in distribution from the low grades to the higher in the succeeding parthenogenetic generations, and the persistent higher defectiveness of the black-eyed wasps.

Significant in the distribution curve is the depression at grade 5 which is found in all generations, and is fully equalled only by the drop at grade 1 in F_{10}. Apparently the *D* sons fall largely to the left of this mode and

the *d* sons to the right (WHITING 1924, p. 35). That the number of minor factors for defect are progressively accumulating in the *d* wasps is evidenced by the fact that, whereas grade 8 rises distinctly in each generation

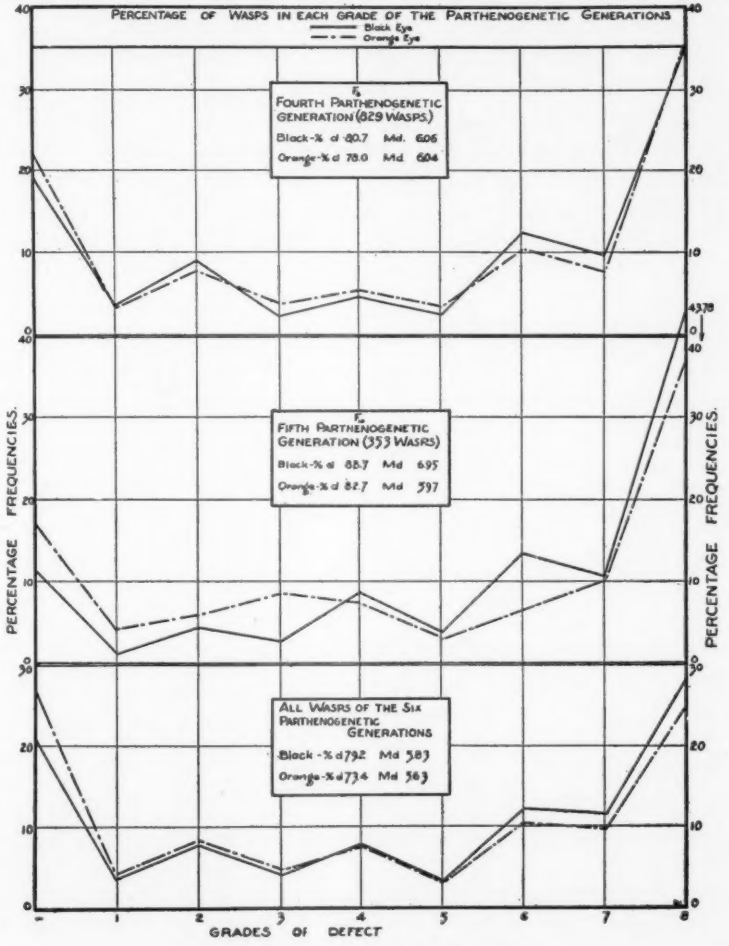

PERCENTAGE OF WASPS IN EACH GRADE OF THE PARTHENOGENETIC GENERATIONS
Black Eye
Orange Eye
F6
FOURTH PARTHENOGENETIC GENERATION (829 WASPS.)
Black-% d 80.7 Md. 606
Orange-% d 78.0 Md. 604
F5
FIFTH PARTHENOGENETIC GENERATION (353 WASPS)
Black-% d 88.7 Md 695
Orange-% d 82.7 Md 597
ALL WASPS OF THE SIX PARTHENOGENETIC GENERATIONS
Black -% d 792 Md 583
Orange-% d 734 Md 563
PERCENTAGE FREQUENCIES.
GRADES OF DEFECT
43.78

DIAGRAM 6b.—A continuation of diagram 6a.

(except as explained for F_{12}), grades 6 and 7 show no progress. In the *d* wasps without the minor factors (stock 3) the mode for the males was at 7 (compare diagram 1).

It seems clear from all this that if those wasps that do not possess the factor *d* could be eliminated, the remaining ones would show a higher-defective condition than stock 3. This is accomplished in the next experiment.

CROSS OF THE HETEROZYGOUS FEMALES AND PARTHENOGENETIC MALES

Grade-8 virgin females were now isolated from F_{11} and mated with grade-8 males from F_{10} and F_{12}. Seven such crosses were made, but five of the resulting lines were discarded in the course of the experiment. Only the last two lines are here discussed. Compare table 5 and diagram 7.

TABLE 5

Progenies of crosses between parthenogenetic males from generation 12 and heterozygous females from generation 11. All selected high-defectives.

Line 6. Leading to stock 16

GENERATION	SEX	GRADE OF DEFECT									TOTAL
		0	1	2	3	4	5	6	7	8	
First generation	♂♂	1	0	0	2	1	1	2	3	7	17
	♀♀	3	1	0	0	0	3	1	10	11	29
Second generation (No. 191)	♂♂	0	0	0	1	2	1	3	16	63	86
	♀♀	0	0	0	0	0	0	1	3	26	30
Third generation (progeny of No. 191)	♂♂	0	0	1	2	1	4	6	32	83	129
	♀♀	1	2	0	1	2	3	13	46	125	193

Line 7. Leading to stock 15

GENERATION	SEX	GRADE OF DEFECT									TOTAL
		0	1	2	3	4	5	6	7	8	
First generation	♂♂	0	0	0	1	2	0	2	3	6	14
	♀♀	0	0	0	0	1	1	4	8	20	35
Second generation (No. 181)	♂♂	0	0	0	0	0	0	1	9	43	53
	♀♀	0	0	0	0	0	0	0	3	34	37
Third generation (progeny of No. 181)	♂♂	0	1	0	0	3	3	16	90	313	426
	♀♀	1	0	1	1	1	8	10	72	278	372

Line 6

Culture T172 produced 17 males and 29 females. One male and three females were type, but the latter were dwarf, indicating that there was

insufficient food. The average of all the defectives was 7.00. From this culture two grade-8 females mated with their brothers produced widely different averages. T189 gave the following:

	Number	*Mn*	*Percent d*
Males	37	5.19	78.38
Females	43	6.76	87.68

T191 averaged much higher:

	Number	*Mn*	*Percent d*
Males	86	7.61	100.00
Females	30	7.83	100.00

T189 was discarded. From T191 five cultures were made that gave fairly uniform results. Their averages follow:

	Number	*Mn*	*Percent d*
Males	129	7.41	100.00
Females	193	7.40	99.48

The one type female was a dwarf and should be eliminated, thus bringing the percentage of defectives up to 100. These wasps are all black-eyed and are preserved as stock 16.

Line 7

Culture T173 gave 14 males and 34 females, all defective, the males averaging 6.75, the females 7.23. A mating from this culture (T181) gave offspring as follows:

	Number	*Mn*	*Percent d*
Males	53	7.79	100.00
Females	37	7.92	100.00

Fifteen grade-8 females from this culture were bred and gave quite uniform averages. They are as follows (diagram 7):

	Number	*Mn*	*Percent d*
Males	426	7.64	100.00
Females	372	7.62	99.73

As in line 6 there is one type female that should be disregarded because she is a dwarf. The wasps of this line are orange-eyed.

The progeny of T181 has been retained as the basis of a new stock, number 15.

In comparing this stock with the original line B of stock 3, it should be noted that in this final strain the difference between males and females is negligible; hence, comparison should be between males. The difference in favor of stock 15 (for males) is: Mn, $1.61 \pm .036$; percent d, $2.53 \pm .17$.

Equally significant is the change in the distribution curve (compare diagrams 1 and 7). The mode in stock 15 is at grade 8 and the curve disappears almost completely at grade 5. This probably represents a combination of all, or nearly all, the factors for defect.

BACKCROSS WITH STOCK 14

Four backcrosses of type males from the parthenogenetic generations with low-grade females of stock 14 were made, partly to serve as a control

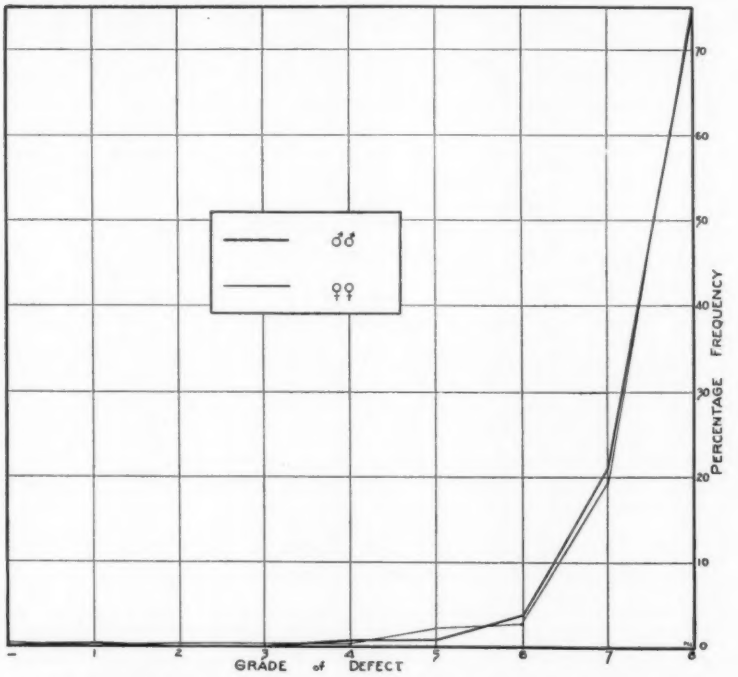


DIAGRAM 7.—Distribution with respect to grade of defect, of progeny resulting from cross between F_{11} heterozygous females and F_{12} parthenogenetic males.

to the high-defective cultures, and also to discover the genetic constitution of the type individuals of the parthenogenetic generations. Large numbers were bred from two cultures that differed conspicuously. In the second generation F141 produced 73.96 percent defectives and F171 gave 58.59 percent defectives. The Mn was 3.39 and 2.41, respectively. In the third generation, presumably as the result of selection, the averages dropped

much lower, as shown in table 6, and the difference between the two lines remained equally striking. In seeking an explanation of this difference the parents of the two lines were investigated. The female used in F171 comes from a line of stock 14 that is considerably lower than that from which the F141 female was taken. Besides, there had been a conspicuous drop in the parthenogenetic males of F_{12} below those of F_{10}, and the male parent of F171 was taken from the former, while F141 was derived from the latter.

TABLE 6

Progenies of backcrosses between low-grade parthenogenetic males from generations 10 or 12 and low-grade females from stock 14.

GENERATION	SEX	GRADE OF DEFECT									TOTAL
		0	1	2	3	4	5	6	7	8	
First generation....	♂♂	17	10	7	12	11	4	2	4	0	67
	♀♀	18	5	10	8	6	8	4	1	0	60
Second generation..	♂♂	128	48	55	28	26	14	16	3	0	318
	♀♀	71	21	36	29	15	16	21	2	0	211
Third generation	♂♂	135	38	47	46	34	20	9	5	0	334
from F141.....	♀♀	65	15	35	26	19	13	5	0	0	178
Third generation	♂♂	287	64	75	36	42	17	8	3	0	532
from F171.....	♀♀	214	55	57	31	23	8	4	1	0	393

			From F141	From F171
Averages of third generation	Mean defect	♂♂	1.78	1.21
		♀♀	1.88	1.08
	Percent defective	♂♂	59.59	46.06
		♀♀	63.49	45.55

The wasps from this backcross average much lower than the wasps of the original stock 14. When we consider that the females of stock 14 used in these cultures were taken from stock 14 after it had shown the effect of selection (diagrams 3 and 4) the result is somewhat anomalous. However, there seems to have been a sudden drop in at least one line of stock 14 as suggested by the low averages of F_{11} and F_{12}, and as seems almost demonstrated by an analysis of the males resulting from the first generation of the backcross. These males are, of course, pure stock 14 except as affected by patrocliny which in this case is probably a negligible factor (P. W. WHITING 1924, p. 40; A. R. WHITING 1925). Their curve as

plotted from table 6 is very similar to the first cultures of stock 14 (diagram 8, F_1 ♂♂).

It is noteworthy that of 2093 wasps examined from this backcross, not one was defective grade 8.

LINKAGE OF ORANGE WITH DEFECT

In the cross of stock 14 by stock 3 the factor *o* came in with the factor for high defect, *d*, and *O* came in with the factors for low defect, *l*, *m*, *n*. The males of the parthenogenetic generations were tabulated to show any

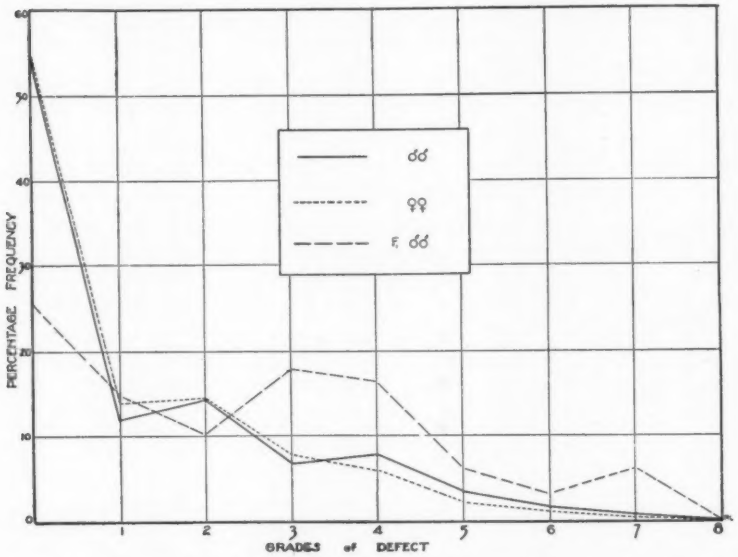


DIAGRAM 8.—Progeny of backcross with stock 14, showing the percentage of wasps in each grade.

relation between eye color and defective condition. The numbers and distribution of defectives in each eye-color group are shown in table 4, and the facts are illustrated graphically in diagram 6. Of 3484 wasps examined, 1740 were orange-eyed and 1744 black-eyed, indicating that there is no significant difference in viability. But the ratios and averages of defectives differ conspicuously and consistently in all generations. The black-eyed are more defective. This means that one or more of the factors *l*, *m*, *n*, are linked with eye color. This linkage has already been pointed out by WHITING (1924, p. 45).

There is no conclusive evidence of crossing over. In F_6 and F_8 the differences are smaller than in the other generations, but there is no reversal of the difference except in the average of defectives (Md) of F_6. This is probably accidental. In F_6 there were 301 orange-eyed wasps, 225 of which were defective, and 300 black-eyed wasps, 237 of which were defective; but the 225 defective orange averaged slightly higher than the 237 black. A very few of the 76 orange that were somatically free from defect might have lowered the Md if they had become defective grade 1. In this connection it may be well to emphasize that these wasps are all genetically defective and hence type individuals do not represent a class by themselves; they are merely an extreme variation from the mean, caused, as the other variations, by external or internal environmental conditions, and they are classed together in one group only because there is no external indication of how far this tendency toward extreme variation goes (P. W. WHITING 1924, p. 49). If the curve were complete it would probably taper off to the left. In a word, type individuals represent an accumulation of extreme variants that cannot be accurately classified. For this reason the Mn should be considered more significant than the Md; it takes into account the extreme variant called type, whereas the Md does not include type specimens at all.

LINKAGE RELATIONS OF SOOTY CHEST

Sooty chest is a highly variable character present in stock 14. Wasps of stock 3 raised at 30° C have a clear yellow chest, whereas in stock 14 the color varies from jet black to yellow. For purposes of study three grades are distinguished: (a) the type color, yellow, labeled s−; (b) intermediate conditions, s_1; and (c) pure black, s_2. Variation is affected by environment during development in a manner similar to that of vein defect, that is, low temperature and insufficient food tend to produce more sooty chest.

All wasps of the parthenogenetic generations F_4 to F_{12} were examined for chest color and the results tabulated. It was found that there is a positive correlation between black eye and sooty chest, which, though not large, is so constant that it must be significant. The following percentages are based on 1281 wasps in each group, all male, and of course bred under identical conditions. The black-eyed wasps examined have an average of $81.34 \pm .74$ percent sooty chest, and the orange-eyed wasps $77.53 \pm .79$ percent. The difference is 3.81 ± 1.08 percent. While the probable error is somewhat large, the following table shows that the ratios of these percentages remained fairly constant during the five generations examined.

Generation	Percentage of sooty chest		Ratio of orange to black
	Orange eye	Black eye	
F_4	81.23	83.40	.954
F_6	80.40	84.00	.952
F_8	74.75	81.41	.913
F_{10}	73.21	75.67	.966
F_{12}	74.70	77.27	.952

Since orange eye is not a variable character the correlation cannot be accounted for by any theory of concomitant variation, as might possibly be done in the case of sooty chest and defective vein, both of which are variable characters. The above data indicate one of two things: either there is linkage between one of the factors for sooty chest and the factor for eye color, or black eye is itself a factor for sooty.

The correlation between chest color and vein defect is quite marked. Table 7 summarizes the data, based on the same wasps as above.

TABLE 7

GRADE	NUMBER OF WASPS				PERCENT SOOTY CHEST
	Yellow		Sooty	Total	
	S−	S+	S+		
0	6	41	486	533	91.19±0.82
1	1	5	83	89	93.26±1.80
2	3	17	176	196	89.80±1.46
3	0	6	98	104	94.23±1.54
4	9	15	128	152	84.22±2.00
5	6	6	61	73	83.56±2.91
6	5	37	250	292	85.62±1.38
7	11	54	218	283	77.03±1.65
8	55	250	535	840	63.69±1.12
Totals	96	431	2135	2562	79.43±1.04

There is an increase of sooty chest from 63.69±1.12 percent in wasps of grade 8 to 92.24±.59 percent in wasps of grade 3 to type. The largest percent of sooty chest is at grade 3, but this mode is probably not significant, its difference from type being only 3.04±1.73 percent.

Since sooty chest was introduced into these wasps in combination with factors for low-grade defect, the correlation here found is all the more significant. The conclusion seems justified that there is a genetic linkage between some of the factors for defect and those for sooty chest.

SUMMARY

1. Breeding from individuals selected for opposite extremes of the variable character, defective venation, in a well-established stock of

Habrobracon juglandis, through nineteen generations in one direction and twenty-two generations in the other, has produced no significant difference in the progeny of the two lines.

2. A similar selection experiment, less rigid in method, in another stock that had not previously been inbred, resulted in an immediate change in the direction of selection, the mean defect after ten generations being increased nearly three-fold.

3. By breeding one defective stock to another, the various factors for defect can be concentrated, and they have a cumulative effect.

4. The haploid males of this insect furnish a unique and convenient method for the analysis of heterozygous females; a method that has special value in the case of factors that govern a variable character.

5. Application of the principles named under 3 and 4 has resulted in the creation of two new stocks (Nos. 15 and 16) showing defect in practically all individuals when the usual environmental conditions are maintained, and throwing an average defect of 7.64 as against 5.81 for the highest average previously attained. Expressed in percentages this is an increase from 77.2 percent to 95.5 percent.

6. The factor for orange eye is linked with one or more of the minor factors for defect.

7. There is a significant correlation between black eye color and sooty chest.

8. Amount of defect is negatively correlated with sooty chest, suggesting linkage of some of the factors for defect and those for sooty chest.

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THE EFFECTS OF UNEQUAL CROSSING OVER AT THE BAR LOCUS IN DROSOPHILA[1]

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INTRODUCTION

If one thinks of mutations as being simply inherited changes, it becomes necessary to distinguish changes that involve whole chromosomes (e.g., non-disjunction or tetraploidy), changes that involve several adjacent genes (deficiencies and duplications), and what have been called "point-mutations" or "gene-mutations." Probably this last type includes quite diverse processes. It is therefore important to collect information as to the nature of specific examples of mutation. For this purpose it will commonly be necessary to work with a frequently recurring mutation. Only one frequently mutating gene has hitherto been discovered in Drosophila, namely, bar. Crossing over has proved to be the key to the mutation behavior of bar, as will be shown in the present paper. The case appears not to be, strictly speaking, a point-mutation after all, but a new kind of section-mutation, in which the section concerned is so short as to include only a single known gene, and in which unequal crossing over furnishes the mechanism for bringing about the new types.

HISTORICAL

In 1914 TICE (1914) found a single male of *Drosophila melanogaster* that had narrow eyes (see figures 1 and 2). The new type, called bar (or "barred"

[1] Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

LEGEND FOR PLATE 1

FIGURE 1.—Homozygous bar female.
FIGURE 2.—Bar male.
FIGURE 3.—Bar-over-round female.
FIGURE 4.—Female homozygous for round, that was obtained by reversion.
FIGURE 5.—Male that carries round, obtained by reversion.
FIGURE 6.—Double-bar male.
FIGURE 7.—Homozygous infrabar female.
FIGURE 8.—Infrabar male.
FIGURE 9.—Infrabar-over-round female.
FIGURE 10.—Double-infrabar male.

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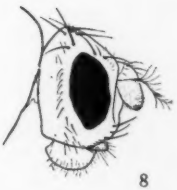

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in the earlier literature), was found to depend on a sex-linked gene located at 57.0 in the X chromosome. It was further found that the bar character is dominant, in the sense that females carrying one bar gene have eyes distinctly different from the wild-type or "round" eye (figure 3). Because of this dominance the type has been extensively used in linkage experiments. MAY (1917) reported that the bar gene occasionally reverts to normal (figures 4 and 5)—a process that has more recently been extensively studied by ZELENY (1919, 1920, 1921). ZELENY found that the frequency of reversion is variable, but in many stocks is such that about 1 in 1600 offspring from a pure bar stock receives a not-bar, or round, allelomorph. ZELENY also concluded that the reversion probably occurs chiefly (or perhaps exclusively) in females. His argument, based on the sex ratio found among reverted individuals, is not as convincing as the direct tests that will be described in this paper, and which verify his conclusion. ZELENY also found that homozygous bar gives rise to a new and more extreme allelomorph of bar, that he has called "ultra-bar." For reasons that will be developed in this paper, I prefer to call it "double-bar." The eyes of double-bar are distinctly smaller than those of bar (figure 6). ZELENY has shown that the type is more strongly dominant over round than is bar, and also that double-bar is largely dominant over bar.

ZELENY likewise found that homozygous double-bar stocks revert to round with a frequency not very different from that of homozygous bar stocks, and that double-bar occasionally mutates to bar; that is, it can go all the way back to round at one step, or it can give bar, which, in turn, is capable of reverting to round. ZELENY has argued that the three types, round, bar, and double-bar, have the same characteristic properties, regardless of their origin. The round eye of reverted bar is indistinguishable from wild-type; bar derived from double-bar does not differ from the original bar, etc. This point will be considered in more detail in a later section.

STURTEVANT and MORGAN (1923) showed that double-bar over bar[2] also gives rise to round-eyed individuals. They reported three reversions from this combination and three from homozygous bar. In all cases the mothers had been heterozygous for forked,[3] which lies 0.2 units to the left

[2] In this paper the constitution of heterozygotes will be expressed as above in order to avoid circumlocution or indefiniteness. "Double-bar over bar" is to be understood as: "carrying double-bar in one X chromosome and bar in the other X chromosome."

[3] Forked is a recessive bristle modification. Locus 56.8 in the X chromosome (see MORGAN and BRIDGES 1916).

of bar, and for fused[4] which lies 2.5 units to the right of bar. All six reversions represented crossovers between forked and fused, though the total forked fused crossovers constituted less than 3 percent of the number of individuals examined. STURTEVANT and MORGAN also reported that experiments in which bar entered only through the males had failed to give any reversions, though no numerical data were reported. The present paper is based on the results of a more detailed study of the relations first shown by STURTEVANT and MORGAN (1923).

MUTATIONS AND CROSSING OVER

The results from homozygous bar females, that were reported by STURTEVANT and MORGAN, were from females of the constitution $\frac{+Bf_u}{fB+}$. A more efficient type of experiment is that in which females of the constitution $\frac{+B+}{fBf_u}$ are mated to forked bar fused males. Table 1 (first row) shows the results obtained from an extensive series of this type. In the second row of table 1 are given the results from mating a few females of the above constitution to forked fused males.

TABLE 1

$\frac{B}{fBf_u}$ ♀ × fBf_u ♂ *(1st row) or* ff_u ♂ *(2nd row).*

TYPE OF MALE USED	BAR								TOTAL	ROUND					DOUBLE-BAR	
	♀				♂					♀		♂			♀	♂
	0*		1		0		1			f_u	f	+	f_u	f	f	f
	+	ff_u	f_u	f	+	ff_u	f_u	f								
fBf_u	5413	3749	94	140	5218	4160	93	124	18991	0	2	1	2	1	1	1
ff_u	374	359	6	6	352	324	10	7	1438	1	0	0	1	0	0	0
Total.....	5787	4108	100	146	5570	4484	103	131	20429	1	2	1	3	1	1	1

* In this and the following tables "0" signifies non-crossover classes; "1," classes resulting from crossing over in the first region, etc.

The mutant females that appeared in these experiments were, of course, all heterozygous for whichever allelomorph (bar or round) they received from their fathers, but in tables 1 to 18 this paternal gene is ignored and the females classified according to the maternal gene. In all doubtful cases (including both double-bars), the classification was checked by raising offspring from the mutants, since there is sometimes difficulty in

[4] Fused is a recessive venation character. Its locus is at 59.5 in the X chromosome (see MORGAN and BRIDGES 1916, LYNCH 1919).

classifying single individuals, a difficulty that can be removed by examination of a series of specimens of a given constitution.

It will be observed that seven of the eight reversions, and also both of the double-bars, occurred in gametes that came from crossing over between forked and fused, though the total forked fused crossovers constituted only 2.4 percent of the population. The one exceptional case, a wild-type male, is not above suspicion of having arisen through contamination rather than reversion of bar. That he was really round-eyed was proven by tests. Only 4 other exceptions to the rule that mutation in this locus is accompanied by forked-fused crossing over have been met with in the work here reported. These will be discussed separately later.

On the basis of these results we may formulate the working hypothesis that both reversion and the production of double-bar are due to *unequal crossing over*. If we suppose that, in a female $\frac{+B+}{f\,B\,f_u}$, crossing over occurs in such a way that the respective points of interchange lie to the left of the bar locus in one chromosome, but to the right of it in the other one, there will result chromosomes of the constitution $fBB+$ and $+f_u$ (or $f+$ and $+BBf_u$). The hypothesis is that reverted round is simply *no-bar*, and that double-bar is *BB*,—this being the reason for abandoning ZELENY'S name, ultra-bar.

This hypothesis makes reverted round and double-bar complementary crossovers, and they should accordingly be produced with equal frequency. Table 1 agrees with ZELENY'S more extensive data in showing that round is apparently far more frequent than double-bar; but such a result was to be expected for two reasons. Double-bar is not as viable as round, so that fewer of the double-bar mutant individuals would be expected to survive; and double-bar is not always clearly distinguishable from bar, so that some mutant individuals are probably overlooked, while it is not likely that any reversion is overlooked through difficulty of classification.

The double-bar over bar experiments reported by STURTEVANT and MORGAN (1923) can be interpreted in the same way: the reversion is here due to unequal crossing over just as in homozygous bar. In the earlier cultures of the experiments previously reported, only the reversions were classified for forked and for fused. Two of the reversions were in such incompletely classified cultures. Table 2, including all the double-bar over bar data for which complete counts are available, contains one of the previously reported reversions and one new one. This table includes only the male offspring, since the *BB* derived from the fathers rendered the classification of the females uncertain.

The process of unequal crossing over might be expected to give rise to triple-bar from the females that are double-bar over bar. No individual that could be so identified was obtained, though several specimens with very small eyes were tested. All those that were fertile proved to be double-bar. Apparently triple-bar is either inviable or sterile. This problem will be discussed again below.

TABLE 2

$\frac{B}{f\,BB\,f_u}$ ♀ × $f\,BB\,f_u$ ♂.

B OR *BB* ♂				TOTAL	ROUND ♂
0		1			
+	$f\,f_u$	f_u	f		f
3933	2741	62	73	6809	2

My own experiments with homozygous double-bar have not yielded any mutations, probably because I have found this type hard to breed and have therefore not obtained large numbers of offspring. It may be recalled that ZELENY has obtained both bar and round from such females, but not in experiments in which forked and fused were present. In table 3, showing the data I have obtained, only males are recorded, for the same reason as in table 2, and also because the females could not be classified for fused.

TABLE 3

$\frac{f\,BB}{BB\,f_u}$ ♀ × $f\,BB$ ♂.

BB ♂				TOTAL
0		1		
f_u	f	+	$f\,f_u$	
627	956	37	24	1644

In the case of double-bar over round, bar should be produced by any crossover between the two bars of the double-bar chromosome. This event might seem less unlikely to occur than the type of unequal crossing over invoked in the preceding experiments; and both of the chromosomes resulting from such crossing over should yield bar, whereas in the preceding cases, a given crossing over must always have yielded chromosomes bearing two different kinds of bar allelomorphs. It is accordingly in

agreement with the hypothesis that tables 4 and 5 show a higher percentage of mutation than do tables 1 and 2. Two non-disjunctional individuals (a ff_u♂ and a $\frac{+\ +\ +}{f\ BB\ f_u}$ ♀) have been omitted from table 5.

TABLE 4

$\frac{BB}{ff_u}$ ♀ × ff_u ♂.

SEX	DOUBLE-BAR AND ROUND						TOTAL	BAR	
	0		1		2				
	BB	ff_u	f_u	*f BB*	BBf_u	*f*		f_u	*f*
♀	1174	784	1	1	18	30	2018	0	1
♂	993	943	1	1	14	19	1971	1	1

No mutations are to be expected from females that are heterozygous for bar and for round $\left(\frac{B}{+}\right)$, since crossing over can not produce any new combination. The results of the tests are shown in tables 6 and 7, and are in agreement with this expectation. One non-disjunctional female $\left(\frac{+\ +\ +}{f\ B\ f_u}\right)$ was also produced in this series.[5]

TABLE 5

$\frac{+}{f\ BB\ f_u}$ ♀ × ff_u ♂.

SEX	DOUBLE-BAR AND ROUND						TOTAL	BAR	
	0		1		2				
	+	*f BB* f_u	BBf_u	*f*	f_u	*f BB*		f_u	*f*
♀	464	392	0	1	14	23	894	1	0
♂	495	406	0	0	6	10	917	1	1

L. V. MORGAN (1922) has described a race of *D. melanogaster* in which the two X chromosomes of the female are attached to each other, so that such a female gives 100 percent non-disjunction. This race, and others

[5] This series also produced one female that was wild-type in appearance. Such a female would be either a double crossover—which is not at all probable in such a short chromosome section, or a non-crossover reversion of bar. This female was mated to an unrelated bar male, and produced 124 bar-over-round daughters and 96 round-eyed sons; but none of the sons showed either forked or fused. This result must mean that the exceptional female was due to contamination.

separate in origin but having the same peculiarity of attached X's, have been used to test the mutability of bar in the male. If a round-eyed female with attached X's is crossed to a bar male, all the sons get their X chromosomes from the bar father and accordingly furnish a direct test of the

TABLE 6

$\frac{+}{fB\,f_u}$ ♀ × $f f_u$ ♂.

SEX	BAR						TOTAL
	0		1		2		
	+	$f\,B\,f_u$	$B\,f_u$	f	f_u	$f\,B$	
♀	331	276	0	0	12	13	632
♂	316	265	0	1	10	11	603

mutability of bar in males.[6] A total of 10,079 bar males has been observed from such matings, with no rounds or double-bars. There was, however, one other male that had an eye intermediate between bar and round. This new type, called infrabar (figures 7, 8, 9) has been shown to represent a new allelomorph of bar. Its somatic appearance will be described in more detail later in this paper.

TABLE 7

$\frac{B}{f f_u}$ ♀ × $f f_u$ ♂.

SEX	BAR						TOTAL
	0		1		2		
	B	$f f_u$	f_u	$f\,B$	$B f_u$	f	
♀	1792	1356	3	7	36	41	3235
♂	1523	1334	4	3	26	36	2926

Tests soon showed that infrabar behaved as a single unit in inheritance. Bar can not be recovered from it, and it shows the same linkage relations as bar. The convincing proof that it represents a modification of the bar gene will appear below.

Homozygous infrabar behaves like bar in that it reverts to normal, and also produces a new and more extreme type, double-infrabar (figure 10),

[6] Occasionally the attached X's separate, and a regular son carrying a maternal X is produced. In the present series of experiments this source of error was eliminated by having the two maternal X's differ from the paternal one in at least one other mutant gene besides bar.

analogous to double-bar. As shown in table 8, both these events are again associated with forked-fused crossing over.

Of the three double-infrabar individuals, one was sterile, and one was accidentally lost, but the constitution of the other was established by breeding tests. The type was also obtained in another experiment; and its appearance and mutation behavior will be described later. This series shows the same excess of rounds over double forms as did the bar series, and the explanation is doubtless the same. The difficulty of separation is even greater here, owing to a greater variability in eye size (see below).

TABLE 8

$\frac{B^i}{fB^if_u}$ ♀ × $f\,B^i\,f_u$ ♂.

SEX	INFRABAR					ROUND		B^iB^i	
	0		1		Total				
	+	ff_u	f_u	f		f_u	f	f_u	f
♀	4607	3606	94	142	8449	0	5	0	1
♂	4341	3905	97	106	8449	2	11	2	0

Bar-over-infrabar females of three different constitutions have been tested. They have produced rounds, and also a new double type that had eyes only very slightly larger than those of double-bar. These must evidently have bar and infrabar in the same chromosome. Tables 9, 10 and 11 show these results. In most cases the fathers of these cultures did not

TABLE 9

$\frac{f\,B^i}{B\,f_u}$ ♀ × *various* ♂♂.

BAR AND INFRABAR MALES					DOUBLE TYPE	
0		1		Total	♂	
f_u	f	+	ff_u		+	ff_u
623	682	26	18	1349	1	1

carry fused; for this reason the females, among which no mutants were detected, are not listed. The forked fused mutant male was sterile, but the constitution of the other was tested. All the experiments here described, on "bar-infrabar" (figure 10), concern this mutant gene. The stability of this new type in the male has also been tested. Matings of bar-infrabar males to attached-X females (also differing in at least one other sex-linked

gene) have produced 9042 non-disjunctional sons,—all of them bar-infrabar.

TABLE 10

$\frac{B^i}{f\,B\,f_u}$ ♀ × $f\,B\,f_u$ ♂.

SEX	BAR AND INFRABAR				Total	MUTANTS
	0		1			
	+	$f\,f_u$	f_u	f		
♀	497	415	10	18	940	0
♂	505	476	15	16	1012	0

The double-type male of table 11, which resembled those from table 9, was tested. His descendants are discussed below under the name "infrabar-bar."

TABLE 11

$\frac{B}{f\,B^i\,f_u}$ ♀ × $f\,B^i\,f_u$ ♂.

SEX	BAR AND INFRABAR				Total	ROUND		DOUBLE TYPE
	0		1					
	+	$f\,f_u$	f_u	f		f_u	f	f
♀	2445	2203	59	74	4781	0	1	0
♂	2365	2347	64	71	4847	1	0	1

These new double types have made it possible to devise crucial tests of the theory of unequal crossing over, which may now be described. The first test made was that of bar-infrabar over round. Tables 12 and 13 show the results obtained. In addition one culture of this series gave two wild-type females, one in the first count and another in the last count,

TABLE 12

$\frac{f\,BB^i}{f_u}$ ♀ × $f\,f_u$ ♂.

SEX	BAR-INFRABAR AND ROUND						Total	BAR	INFRABAR
	0		1		2				
	f_u	$f\,BB^i$	BB^i	$f\,f_u$	+	$f\,BB^i\,f_u$		$f\,f_u$	+
♀	303	394	4	0	14	8	723	0	1
♂	321	308	0	2	7	3	641	1	1

ten days later. These females give rise to the same difficulties as did the wild-type obtained in the series reported in table 7. One of them was tested, and gave unexpected results. The mother had carried vermilion in her forked fused X, and the father had also been vermilion forked fused. The tested exceptional female was found to carry one wild-type X and one forked fused X, but did not carry vermilion at all. Under the circumstances it is open to question whether these two exceptional individuals were not due to contamination. They will be discussed again later.

TABLE 13

$\frac{BB^i}{f\ f_u}$ ♀ × $f f_u$ ♂.

SEX	BAR-INFRABAR AND ROUND							BAR	INFRABAR
	0		1		2		Total		
	BB^i	$f f_u$	f_u	$f\ BB^i$	$BB^i f_u$	f		f_u	f
♀	2549	1684	3	1	43	74	4354	0	0
♂	2322	1857	5	8	48	63	4303	2	4

Tables 12 and 13 show that bar and infrabar can both be recovered from bar-infrabar. It appears then that in the double form the individual elements maintain their identity. Even more important, however, is the indication that they maintain their sequence in the chromosome. As shown in table 9, the bar-infrabar first came from the combination $\frac{f\ B^i}{B f_u}$, as a not-forked not-fused male. If the two elements of double forms are arranged in the same linear series as the rest of the genes, this result must mean that the bar now lies to the left of the infrabar. This supposition is entirely borne out by tables 12 and 13, which are experiments of the usual type used to establish sequence of genes. All the 9 single types recovered agree with the supposed sequence.

TABLE 14

$\frac{BB^i}{f\ B^i f_u}$ ♀ × $f\ B^i f_u$ ♂.

SEX	BB^i AND B^i							B^iB^i
	0		1		2		Total	
	BB^i	$f\ B^i f_u$	$B^i f_u$	$f\ BB^i$	$BB^i f_u$	$f\ B^i$		f
♀	850	702	3	3	17	28	1603	0
♂	867	860	4	2	22	23	1778	1

Bar-infrabar has also been tested against infrabar (table 14).

Here again there is an opportunity for the production of a triple form,—but since the corresponding round did not appear, its absence is not significant. The one double-infrabar confirms the sequence of the component parts of bar-infrabar. It was tested, and all the results from double-infrabar reported below were obtained from flies descended from it.

Examination of the data in table 11 shows that the double form obtained there must have had infrabar to the left of bar; that is, it was infrabar-bar instead of bar-infrabar. The tests made with it appear in table 15.

TABLE 15

$\frac{f\ B^{i}B}{f_{u}}$ ♀ × $f f_{u}$ ♂.

SEX	INFRABAR-BAR AND ROUND						Total	BAR	INFRABAR
	0		1		2				
	f_{u}	$f\ B^{i}B$	$B^{i}B$	$f f_{u}$	+	$f\ B^{i}B f_{u}$		+	$f f_{u}$
♀	483	469	1	1	15	15	984	2	0
♂	478	408	1	9	17	15	928	0	1

These results show, in fact, that the sequence is infrabar-bar as supposed. Except for this difference the mating is the same as in table 12. It will be observed that the two not-forked not-fused single types in that table were both infrabar, while the two obtained here were bar; the one forked fused there was bar, here it was infrabar. There is a total of 13 single-type mutants in tables 12 to 15, all of them agreeing in indicating that the two elements of double types maintain not only their individuality but their sequence.

The double-infrabar obtained in table 14 has been tested against round (tables 16 and 17).

TABLE 16

$\frac{f\ B^{i}B^{i}}{f_{u}}$ ♀ × $f f_{u}$ ♂.

SEX	$B^{i}B^{i}$ AND ROUND						Total	B^{i}
	0		1		2			
	f_{u}	$f\ B^{i}B^{i}$	$B^{i}B^{i}$	$f f_{u}$	+	$f\ B^{i}B^{i} f_{u}$		+
♀	538	629	1	1	12	17	1198	0
♂	574	505	1	1	13	7	1101	2

The results in these tables show that double-infrabar was correctly identified, and that it behaves as was to be expected, giving infrabar by both kinds of crossing over,—just as double-bar gives bar in both cases.

TABLE 17

$\frac{B^iB^i}{f\ f_u}$ ♀ × $f f_u$ ♂.

SEX	DOUBLE INFRABAR AND ROUND							INFRABAR	
	0		1		2		Total		
	B^iB^i	ff_u	f_u	$f\ B^iB^i$	$B^iB^if_u$	f		f_u	f
♀	1664	1404	3	3	39	37	3150	1	1
♂	1449	1193	3	3	29	42	2719	1	2

Double-infrabar over infrabar has also been tested, in the hope of obtaining triple-infrabar (table 18).

No triple-infrabar was detected; but its absence is not surprising, since the corresponding round occurred only once, and since it is not at all sure that the triple form could be distinguished from the double one.

TABLE 18

$\frac{B^iB^i}{f\ B^if_u}$ ♀ × $f\ B^if_u$ ♂.

SEX	DOUBLE INFRABAR AND INFRABAR*					ROUND
	0		1		Total	
	+	ff_u	f_u	f		f
♀	851	777	25	31	1684	1
♂	665	750	26	29	1470	0

* The double-infrabar and infrabar flies are not entered separately. In the original counts they were separated; but the classification is uncertain at times.

One further type of female was tested, namely, bar-infrabar over infrabar-bar (table 19).

The one mutant infrabar obtained in the series is in agreement with the sequence in which bar and infrabar were supposed to lie in the two X chromosomes of the mother; and this mutant is also the only one yet obtained from a mother carrying a double-type allelomorph in each X, where forked and fused were heterozygous. It therefore serves to complete the demonstration of the relation of crossing over (between forked and fused) to mutation in the bar locus.

The experiment of table 19 was, however, planned for another purpose. It will be seen that in the mother, which was $\frac{B\ B^i}{B^i\ B}$, *equal* crossing over might give rise to new types, namely, double-bar and double-infrabar.

TABLE 19

$\frac{BB^i}{fB^iB\ f_u}$ ♀ × *f* $B^iB\ f_u$ ♂ (*1st row*), *or* *f* f_u ♂ (*2nd row*).

	BB^i AND B^iB									B^i
	♀				♂					♂
	0		1		0		1		Total	*f*
	+	*f* f_u	f_u	*f*	+	*f* f_u	f_u	*f*		
1*	...	...	.	..	95	74	3	4	176	0
2	428	335	8	15	341	319	7	16	1469	1

* Females not counted in the mating to B^iB male.

The first could not be distinguished, in somatic appearance, from the unmutated double types (BB^i and B^iB); but the double-infrabar should be readily detected. Such an individual would be forked. It may accordingly be concluded that none of the 35 forked (not-fused) offspring represented equal crossing over between the halves of the two double-type bar allelomorphs present. It therefore seems probable that crossing over of this kind is not much, if any, more frequent than is that between the two elements of a double-type allelomorph when the other chromosome carries round (tables 4, 5, 12, 13, 15, 16).

Several of the above tables agree with a small series of infrabar over round, heterozygous for forked and for fused, in showing that infrabar lies between forked and fused. It must clearly be either an allelomorph of bar, or bar plus a modifier that lies near bar.

The experiments with bar-infrabar and with infrabar-bar show that these two types both contain infrabar as a unit distinct from bar.

Since bar-infrabar was produced by an unequal crossover that occurred very close to the left of infrabar, it becomes unlikely that a modifier can lie on that side of the bar locus; infrabar-bar furnishes similar evidence that there is no modifier to the right. All the evidence thus indicates clearly that the infrabar gene is really a modification of the bar gene itself.

FREQUENCY OF BAR MUTATIONS

The data presented in tables 1 to 19 have been examined in an attempt to formulate some general statements as to the relative frequency of the

various types of mutation in the bar locus. It is probable that homozygous double types, and double over single show the lowest frequencies of mutation, and that double type over round shows the highest. Both these results might have been expected. There is, however, so much variability among crosses of the same general nature that these conclusions must be accepted with caution. For example, the two largest series are those from homozygous bar (20,438 offspring) and from homozygous infrabar (16,918 offspring). The mechanical conditions should be alike in the two cases, since both represent homozygous single types. Yet from the first there appeared 0.03 percent of reversions, or 1 in each 2920 offspring; from the second there were 0.11 percent, or 1 in 940 offspring. In view of such unexplained differences as this, and in view of the statistical difficulty of determining probable errors for such small percentages, it does not seem profitable to discuss further this aspect of the data, except to note that mutation frequency does not appear to be correlated with frequency of forked-fused crossing over.

THE CROSSOVER VALUES FOR FORKED, BAR AND FUSED

The experiments recorded in tables 1 to 19 include by far the largest series of data yet accumulated for the crossover values of the three loci, forked, bar and fused. These are summarized in tables 20 and 21. In these tables all the mutant individuals have been omitted. Their inclusion would not have affected any of the values appreciably. In table 22 the

TABLE 20

f B f_u crossing over.

TYPE OF FEMALE TESTED	NON-CROSSOVERS	CROSSOVERS		TOTAL
		Region 1	Region 2	
Single type over round	7,193	18	185	7,396
Double type over round	22,937	52	603	23,592
Double type over single	3,279	12	90	3,381
Total	33,409	82	878	34,369
Percentage	97.21	0.24	2.55	

data already published on these loci are included with the above, in order to arrive at final "map values" for the three loci.

On the basis of these data, it seems best to map forked 0.2 unit to the left of bar, that is, at 56.8; and fused 2.5 units to the right of bar, at 59.5, thus making the forked-fused interval 2.7 units.

FACET NUMBER

It has been shown by ZELENY and MATTOON (1915), MAY (1917) and ZELENY (1922) that selection for number of facets is effective in isolating lines of bar flies with high facet numbers or with low. Though no detailed genetic analysis has yet been reported, there is abundant evidence in

TABLE 21

ff_u crossing over.

TYPE OF FEMALE TESTED	NON-CROSSOVERS	CROSSOVERS	TOTAL
Single type over single....	48,966	1290	50,256
Double type over single...	9,717	246	9,963
Double type over double..	3,175	114	3,289
Total.....	61,858	1650	63,508
Percentage.....	97.40	2.60	

these papers that ordinary bar stocks are heterogeneous for modifiers (not in the bar locus) that affect facet number. This is also the impression I have gained from extensive but less exact studies, with numerous crosses involving bar.

TABLE 22

Total linkage data.

LOCI	SOURCE OF DATA	CROSSOVERS	TOTAL	PERCENT
fB	MORGAN and BRIDGES 1916	8	1,706	0.5
	BRIDGES 1917	5	980	0.5
	Table 20	82	34,369	0.2
	Total	95	37,055	0.26
ff_u	WEINSTEIN 1918	200	8,298	2.4
	Table 21	1650	63,508	2.6
	Total "primary" data	1850	71,806	2.58
	MORGAN and BRIDGES 1916	37	1,201	3.1
	Table 20	960	34,369	2.8
	Grand total	2847	107,376	2.65
Bf_u	MORGAN and BRIDGES 1916	222	8,768	2.5
	BRIDGES 1917	46	1,401	3.3
	Table 20	878	34,369	2.5
	Total	1146	44,538	2.57

Another source of variability in facet number is temperature. It was shown by SEYSTER (1919) that high temperature decreases the facet number of bar, and this relation has been studied in great detail by KRAFKA (1920), ZELENY (1923), A. H. HERSH (1924) and R. K. HERSH (1924). These observers have shown that the effect is present, though in varying degree, in double-bar, round, and in various heterozygotes, as well as in bar. Although these studies furnish essential data for any complete analysis of the mode of action of the bar series of allelomorphs upon development, they need not be further discussed here.

The evidence just reviewed indicated that it would be necessary to get stocks as nearly uniform for modifiers as possible, and also to control the temperature, if any reliable data were to be collected as to the relative effectiveness on facet-number of the various combinations of bar and infrabar. Accordingly, a female that was $\frac{fBf_u}{fBB}$,[7] was mated to a round-eyed vermilion female from vermilion stock. The descendants from this mating were inbred (brother-sister pair matings) for seven generations. In each generation a female heterozygous for two of the three bar allelomorphs concerned was mated to a male carrying the third allelomorph. The line was made homozygous for forked; but both vermilion and fused were eliminated. No other selection was practiced. A female of the third inbred generation was mated to an infrabar male, and a daughter that was infrabar over round was mated to a male from the fourth generation. For three more generations the infrabar series was crossed to the inbred line. After the eighth generation the pedigrees are somewhat more complex, but as close inbreeding as was compatible with maintaining four allelomorphic sex-linked genes was continued for six more generations before the facet counts were begun. The other types studied (bar-infrabar, reverted bar, etc.) were all crossed to the inbred stock just described, at least five times (mostly using females to allow crossing over and get as much of the X's uniform as possible) before being used in the counts. This procedure should have resulted in making the various stocks practically alike with respect to modifying genes, and the results obtained are sufficiently consistent to indicate that there was no heterogeneity in major modifiers, though it is still possible to interpret some of the minor differences observed as being due to uneliminated diversity in modifiers.

[7] Derived from the experiments of table 1, so that the *BB* of the following discussion is known to have come from the *BB* male of that table, and to have been derived from homozygous bar by forked-fused crossing over.

The temperature control used was not very exact, but maximum-minimum daily records show that 25°C was maintained to within about ±1°C, and even these deviations were probably of short duration. For the main body of the experiments it has not proved possible to detect any systematic effect of the fluctuations in temperature that did occur. The few experiments in which such an effect is perhaps present will be specified when described.

The facets of the smaller eyes,—up to and including homozygous bar,—were counted directly under the binocular microscope, usually on etherized flies, but in some cases on alcoholic specimens. The eyes larger than this were not found to be workable by this method. Such specimens were killed and cleared in KOH. The surface of each eye was then removed and mounted on a slide. By the aid of a camera lucida a drawing was made, representing each facet by a dot, and these dots were then counted, each dot being marked by a check as counted. In all cases the right eye alone was used.

The main series of data is shown in table 23.

The table shows that homozygous infrabar is about like bar over round in facet number, but the two types can be separated by a peculiarity common to all the larger infrabar and double-infrabar types, namely, a roughened appearance of the eye, due to irregularities in the rows of facets. This peculiarity is not present in bar eyes, and is almost completely recessive in bar over infrabar. In infrabar over round (which is not far from round in facet number) the roughness is variable in extent, and may be not at all evident,—in which case the type can not be distinguished with certainty from homozygous round. In other stocks, where the modifiers are different, it often happens that infrabar over round is regularly conspicuously roughened and is easily distinguishable from round. This roughness of the eyes may be taken as evidence that the infrabar gene is qualitatively different from bar, rather than being merely a fraction of bar.

The table shows that in general when bar and infrabar are both present in an individual, the infrabar produces almost as great an effect in reducing facet number as would another bar, though in the absence of bar the infrabar is far less effective. For example, $\frac{B}{B}=68$, $\frac{B^i}{B^i}=348$; but $\frac{B}{B^i}=74$. And in general, BB^i is practically as effective as BB throughout the table. In two cases the observed differences, though surely not significant, indicate that BB^i is more effective than BB (that is, the combinations

with BB and with B^i). Similar relations are shown in other parts of the table.

TABLE 23

Facet numbers of flies carrying various combinations of bar allelomorphs.

		BB	BB^i	B^iB^i	B	B^i	+
$B\,B$	n	47					
	M	24.96±.30					
	σ	3.03±.21					
$B\,B^i$	n	20	51				
	M	24.10±.35	26.69±.29				
	σ	2.36±.25	3.07±.21				
B^iB^i	n	25	32	27			
	M	26.68±.29	27.91±.36	38.19±.71			
	σ	2.13±.20	2.98±.25	5.48±.50			
B	n	51	86	28	50		
	M	36.43±.30	37.01±.25	38.29±.59	68.12±1.09		
	σ	3.18±.21	3.49±.18	4.64±.42	11.15± .77		
B^i	n	30	53	3	30	18	
	M	41.77±.41	37.79±.52	138.2	73.53±1.29	348.4±12.4	
	σ	3.34±.29	5.62±.37	——	10.52± .92	78.0± 8.8	
+	n	161	70	27	25	25	25
	M	45.42±.24	50.46±.40	200.2± 8.6	358.4± 7.9	716.4± 3.4	779.4± 4.1
	σ	4.57±.17	4.93±.28	66.7± 6.1	58.2± 5.6	24.9± 2.4	30.6± 2.9
♂	n	261	247	40	40	25	25
	M	29.02±.17	29.68±.15	45.98±1.19	91.03±1.76	478.1±13.7	738.8±6.5
	σ	4.02±.12	3.94±.10	11.13± .84	16.61±1.25	101.8± 9.7	48.1±4.6

The most striking relation shown by table 23 is that the relative position of identical genes affects their action on facet number. There are three similar comparisons to be made:

$$\frac{B}{B} = 68.1 \textit{ versus } \frac{BB}{+} = 45.4$$

$$\frac{B}{B^i} = 73.5 \textit{ versus } \frac{BB^i}{+} = 50.5$$

$$\frac{B^i}{B^i} = 292.6^8 \textit{ versus } \frac{B^iB^i}{+} = 200.2$$

[8] This value for $\frac{B^i}{B^i}$ is different from the one of table 23. It is based on a series reared at the same time as the $\frac{B^iB^i}{+}$ with which it is here compared. The difference between this value and that of the table is probably due to temperature.

Since the bar allelomorphs are to be thought of as inhibitors of facet development, it will be seen that this comparison indicates that *two bar allelomorphs lying in the same chromosome are more effective than are the same two allelomorphs when they lie in opposite chromosomes.*

Such an unexpected result must of course be checked up carefully. Only two possibilities of avoiding the above conclusion seem open. The results are due to (1) accidental differences in temperature or modifiers; or (2) the round allelomorph of bar brings about a reduction in facet number just as does bar. Both of these possibilities can be eliminated, as the following paragraphs will show.

There is no temperature effect, since in each case the cultures were reared side by side; and in the case of $\frac{B}{B}$ *versus* $\frac{BB}{+}$, several different tests all gave the same type of result.

If the results are due to accidental modifiers it is scarcely conceivable that these should lie anywhere but near the bar locus, because of the inbreeding to which the stocks have been subjected. As will be shown below, another bar chromosome (derived by mutation from the inbred *BB* of this strain) has been found to give results sufficiently close to the bar used here so that the conclusions as to the effect of position must apply also to the new bar. And two other not-bar ("round") chromosomes have been found to give substantially the same result as the one employed here (see below). These facts eliminate the possibility of explaining the result as due to accidental genetic or environmental differences.

The second possible escape from the conclusion as to the effect of position lies in the assumption of an effect produced by the round allelomorph. This has been tested by determining the effect on facet number of reverted bar and reverted infrabar. Round obtained by reversion from homozygous bar or infrabar stocks cannot carry a normal allelomorph on the view advanced in this paper, unless such an allelomorph is already present in the parent stocks. But such a gene is, almost by definition, not an allelomorph of bar; and in any case cannot be supposed to produce the effects here under discussion, since the $\frac{B}{B}$ and the $\frac{BB}{+}$ would both carry it.

Two different rounds by reversion have been introduced into the inbred stocks, by the same method as used for BB^i and B^iB^i. Care was taken never to use flies carrying one of these reverted rounds in the same culture with the old round of the inbred stock, so that it is certain that these new rounds are really due to reversion,—if not from the supposed source, then from some of the allelomorphs within the inbred stock, since a new

reversion may have occurred during the process of getting the desired modifiers into the reverted round stocks. One of the reverted rounds, called "rev. *B*," came from the homozygous bar experiments of table 1; the other, called "rev. B^i," came from the homozygous infrabar experiments of table 8. The results of these tests are shown in table 24.

TABLE 24

Facet counts to test nature of reversions.

	CHROMOSOME TESTED AGAINST		
	BB	BB^i	Y (i.e., ♂)
Wild-type (control)	45.42±.24	50.46±.40	738.8±6.5
Rev. *B*.....	45.63±.45	45.48±.47	.....
Rev. B^i.....	43.92±.26	46.13±.36	704.4±8.0

These data suggest that the reversions,—especially the more thoroughly tested one from infrabar,—may give slightly smaller eyes than does the wild-type allelomorph. Certainly they do not give larger eyes. And still more certainly, with *BB* or BB^i they do not give as large eyes as do the corresponding types $\frac{B}{B}$ and $\frac{B}{B^i}$. These data eliminate the second possible method of explaining away the effect of the position of the bar allelomorphs on facet number. The conclusion must stand as stated on p. 137.

It seems probable that such an influence of the relative position of genes on their effectiveness in development may be interpreted in terms of diffusion and localized regions of activity in the cell. This idea is, however, scarcely worth elaborating until more evidence is obtained. It may, however, be pointed out that there is another possible application of the hypothesis of a position effect.

It has been shown by BRIDGES (1921) that in triploid individuals the recessive genes brown, plexus and speck do not become dominant to their normal allelomorphs even when two recessive allelomorphs and one dominant are present. Unpublished data collected by BRIDGES (in part verified in an independently arisen series of triploids in my own experiments) show that this relation is a general one for allelomorphs that do not produce an obviously intermediate diploid heterozygote. But BRIDGES (BRIDGES and MORGAN 1923) has shown that a different relation may occur, even for some of the same genes that show the former relation in triploids. In the example referred to, a portion of the second chromosome, carrying the normal allelomorph of plexus (among other genes) has become attached to a chromosome III. It is possible, therefore, to obtain individ-

uals with two complete second chromosomes, each of these carrying the plexus gene, while a normal allelomorph of plexus is present in the section attached to a chromosome III. Such individuals are plexus in appearance, —not as extreme as those in a pure plexus stock, but far more like such a stock than like the ordinary triploids carrying two plexus and one not-plexus allelomorphs. While it is possible that the difference here is due to a different "balance" of modifying genes in the extra section of chromosome II, it seems likely that the effective agent is a difference in position. It is to be remembered that in Drosophila the homologous chromosomes lie closely apposed in somatic divisions (see METZ 1916), so that there is probably a real difference in relative positions in the two cases.

Besides the comparison of round (ultimately from vermilion stock) with round obtained by reversion from bar and from infrabar, two other derived types have been compared (as to facet number) with the corresponding original types.

An infrabar from bar-infrabar over round (table 13) was introduced into the inbred strain by six successive backcrosses, and was then compared with the old inbred infrabar, both types being made heterozygous for double-bar. The control (old infrabar) value is rather lower than the value given in table 23, probably because the temperature ran slightly higher.

Since the difference between the two means is about 4.5 times its own probable error, it is probably significant, but more extensive data will be required to establish this point.

A female of the inbred series that was double-bar over round, mated to a round male, gave rise to one bar male by mutation. This male was mated to double-bar-over-round females, and the resulting double-bar-over-bar daughters were compared with double-bar over the old inbred bar, derived from cultures made up at the same time and put side by side. Here again there is a slight difference from the value of table 23, perhaps due to a temperature difference.

The difference between the means is slightly over 3 times its probable error, and may be considered as doubtfully significant. Here again, more data are needed.

It may be pointed out that in tables 25 and 26 the derived type is presumably the larger in both cases. It is possible that this result is to be correlated with that recorded in table 24, namely, that round by reversion is perhaps smaller than wild-type. Both relations are consistent with the view that there exists a normal allelomorph of bar that has an effect on facet number opposite to, but much weaker than, that of bar; for both

of the derived single types tested were from double type over round, so that these derived forms may really be single type plus normal allelomorph. This possibility requires further experimental investigation.

TABLE 25

Facet counts from $\frac{BB}{B^i}$.

	NUMBER	MEAN	σ	DIFFERENCE OF MEANS
Old B^i.....	40	34.73±.35	3.24±.24	2.05±.46
New B^i.....	36	36.79±.31	2.76±.22	

TABLE 26

Facet counts from $\frac{BB}{B}$.

	NUMBER	MEAN	σ	DIFFERENCE OF MEANS
Old *B*	31	33.10±.33	2.76±.24	1.77±.56
New *B*.....	30	34.87±.45	3.65±.32	

ARE MUTATIONS IN GENERAL DUE TO UNEQUAL CROSSING OVER?

One of the first problems raised by the discovery of the nature of bar reversion is as to how widespread may be the phenomenon of unequal crossing over. One direct test has been attempted, making use of MULLER'S method of testing for the frequency of occurrence of new lethal mutations. Females were made up that carried one wild-type X chromosome and one X with the mutant genes scute (locus 0.0), echinus (5.5), crossveinless (13.7), cut (20.0), vermilion (33.0), garnet (44.4), and forked (56.8). Such females were mated to males carrying all the mutant genes named. In such matings it is possible to detect practically all the crossing over that occurs in the X chromosome, except that to the right of forked (about 13 units). Counts were made from individual females, in order to make sure that they carried no lethals. Forty-one wild-type daughters (non-crossovers) were tested from such matings, to see if the non-crossover X chromosomes carried new lethals. The only lethal that occurred was in a paternal (i.e., scute echinus crossveinless cut vermilion garnet forked) chromosome. Thirty-eight double-crossover daughters and one triple-crossover (i.e., a total of 79 crossings over) were also tested; and again no lethals occurred in any of the maternally derived chromosomes, though there were two doubtful cases of new lethals in the chromosomes derived from the multiple-recessive fathers. While this experiment was done on a

small scale, it furnishes no indication that crossover chromosomes are more likely to contain new lethals than are non-crossovers.

There is, however, another kind of evidence that argues against any general applicability of the unequal-crossing-over explanation of mutation, namely, the cases in which mutations can be shown to have occurred in the X chromosomes of males, since it may be taken as established that crossing over does not occur between the X and the Y of a male.

We have seen earlier in this paper that infrabar arose from bar in a male, and that its later behavior was not in agreement with the view that it represented a quantitative change in the bar gene, as it should if due to unequal crossing over. I have also obtained yellow, a fused allelomorph, and a lozenge allelomorph under the same circumstances, namely, from mothers with attached X's and in experiments where known sex-linked genes were present, so that breaking apart of the attached X's was known not to have occurred.

Unpublished data are available for 5 other cases of the same sort, either from attached-X or from "high-non-disjunction" mothers, as follows: rudimentary (C. B. BRIDGES), a dusky allelomorph (C. B. BRIDGES), a sable allelomorph (E. M. WALLACE), white (L. V. MORGAN), and a new lozenge allelomorph from lozenge (C. B. BRIDGES). In all of these cases, as in that of infrabar, the mutant type first appeared as a single male.

MULLER (1920) reported the occurrence of white as a "somatic" mutation in a male. From a stock in which white was not present he obtained a male with one wild-type eye and one white eye. This male also transmitted white to some (all that were tested) of his daughters. In the same paper MULLER described briefly a mosaic male that was partly yellow, and transmitted the new character to his offspring. Doctor BRIDGES informs me that he has a similar (unpublished) record for yellow. MOHR (1923 a) reports a similar case for a singed allelomorph, though here some of the X-bearing sperms carried singed, while others did not. I have observed two other such cases,—both in *D. simulans*. The mutant types dusky and fused (both corresponding to the types of the same names in *D. melanogaster*) each appeared first in an individual that showed the new character in only one wing; and in each case tests showed that some of the X-bearing sperms carried the new gene, while others did not. In all cases discussed in the last two paragraphs, genetic tests have established the allelomorphism of the new mutant genes to the old ones whose names they bear.

There is thus clear evidence that mutations have arisen in the following sex-linked loci in the germ-cells of males: yellow (3 times), white (twice), lozenge (twice), dusky (twice), fused (twice), singed (once), bar (once). It should be noted that all these loci are among the more mutable ones of Drosophila.

Another class of cases to which the unequal-crossing-over hypothesis is probably not applicable is that in which mutation can be shown to have occurred at some stage other than maturation. A number of such instances are on record for Drosophila. The mosaic males described above are examples, and a longer list of cases for autosomal mutant types and for sex-linked mutations in females could easily be compiled. But since there is evidence that crossing over does very rarely occur at somatic divisions, this evidence can hardly be considered decisive. In the case of certain types of frequently recurring somatic mutations in plants, however, the mutation occurs far too often to make an appeal to somatic crossing over seem plausible. The clearest example of this sort is the variegated pericarp of maize studied by EMERSON (1917) and others, in which a given gene mutates many separate times in a single individual plant.

Mutations are known in which there appeared to be no crossing over in the region concerned,—both in females and in males where crossing over does not normally occur at all. The previously cited cases of mutations in the X chromosomes of males are examples. These may seem to furnish conclusive evidence that mutation need not be accompanied by crossing over. There is, however, one possibility that needs to be considered in this connection.

Recent results (not yet published, but soon to appear) obtained with triploid females (BRIDGES and ANDERSON) and with females having unlike attached X's (ANDERSON, L. V. MORGAN, and STURTEVANT) have shown that crossing over must normally occur when the homologous chromosomes are doubled: that is, in a "four-strand stage" (in diploid females). These results show also that crossing over may occur between only two of the strands at a given level. Now, if it be supposed that *sister* strands may cross over with each other, there will result chromosomes in which no rearrangement of mutant genes has occurred, since sister strands come from the division of one chromosome and will be identical in the genes that they carry. Yet it is conceivable that such crossing over might be unequal, and in such a case might lead to the production of a new mutation that did not appear to be due to crossing over.

The data presented in this paper show that such an event must be extremely rare in the case of bar, since no clear case was found of bar mutation (in a female) unaccompanied by evident crossing over between forked and fused. The few exceptional cases may be accounted for in this way; but, as pointed out when they were described, it seems at least equally probable that all of them are due to experimental errors. We must conclude that sister strands do not cross over with each other; or, if they do, that the crossing over is rarely, if ever, unequal.

It is therefore unlikely that apparent non-crossover mutations in other loci are to be referred to crossing over between sister strands.

"PRESENCE AND ABSENCE" AND QUANTITATIVE VIEW OF MUTATION

It will be observed that the hypothesis advocated in this paper makes bar, double-bar and round by reversion (or infrabar, double-infrabar and round by reversion) represent quantitative variations of the same substance. In the case of bar and round, the hypothesis is the same as the original and most special type of quantitative view, the "presence and absence" hypothesis. But the present scheme differs from the earlier ones in that it is based on definite evidence for the occurrence of unequal crossing over. That is, the mechanism whereby the quantitative differences are brought about is an essential part of the hypothesis. In the preceding section we have seen that there is definite evidence to show that unequal crossing over is not usual in the production of new mutant types. It is especially noteworthy that this evidence was derived in part from the white locus of Drosophila and the variegated locus of maize,—two of the best-known examples of loci that have produced large series of multiple allelomorphs. It is clear, therefore, that the bar case does not furnish support to the idea that mutations in general are quantitative in nature. Even with respect to multiple allelomorphs, where the quantitative view has often been urged, it is obvious that, at least in the cases of white and variegated, the bar evidence does not in any way support that view.

ARE DEFICIENCIES DUE TO UNEQUAL CROSSING OVER?

The "section-deficiencies" described by BRIDGES (1917, 1919) and by MOHR (1919, 1923 b) are probably to be interpreted as due to losses of definite sections of chromosomes. It will be observed that bar reversion has here been treated as due to the loss of a very short section; it may accordingly be described as a deficiency that is too short to show the lethal effect and other properties of the previously described deficiencies.

When the case is stated in this way, the queston at once arises: is it probable that notch and other deficiencies have also arisen through unequal crossing over? If so, the contrary crossover should be a chromosome that was double for a region corresponding to the deficient section. Such a chromosome has never been identified, but it may be doubted if it would be detected even if present. Furthermore, it might well be lethal even in heterozygous females, in which case it would not be capable of detection.

There is evidence that deficiencies may arise in other ways than by unequal crossing over. In at least one case (BRIDGES and MORGAN 1923) the section missing from a second chromosome was found to be present, but attached to a third chromosome. In this case, then, the deficiency can not have been due to unequal crossing over. The first deficiency described, that for forked and bar (BRIDGES 1917), occurred first as a single female that had obtained the deficient X from her father. Here the deficiency arose (either in a male or very early in the cleavage of a female zygote) at a time when crossing over (and bar reversion) does not normally occur. In the case of notch, also, there is evidence that the deficiency may originate at stages other than maturation. LANCEFIELD (1922) records the occurrence of a notch (probably corresponding to that of *D. melanogaster*) in *Drosophila obscura;* the mutation was first detected as two females from a pair mating that gave numerous offspring. In this case the deficiency must have originated in the gonial cells of one parent, unless the two notch females received their notch chromosomes from the father, in which case it is just possible that they came from two sperms derived from a single spermatocyte. But in this case the hypothesis of unequal crossing over remains as improbable as before. I have observed two cases in *D. melanogaster* that represent "somatic" (i.e., not occurring at the maturation divisions) occurrences of notch. In one case three notch females were produced from a single mother. The X's of the mother were attached, and the notch daughters, like all their sisters, did not carry a paternal X. These three females were all sterile, so here it was not possible to demonstrate that the new type was actually notch; but the numerous characters of notch make the identification very probable. The other case also occurred in a line in which the females all had attached X's. A female, from a line with no notch ancestry, was notch in the left wing but not in the right. The offspring showed that this female was, like her mother, heterozygous for several sex-linked genes. These included scute, 3 units to the left of notch, and crossveinless, 10 units to the right of it. Some of the eggs of the mosaic carried notch, but many of them did not.

Furthermore, tests showed that scute and crossveinless were in opposite chromosomes in both types of eggs; that is, the mutation to notch occurred at a cleavage division, and was not accompanied by crossing over between scute and crossveinless. From these three instances we may conclude that the notch deficiency may arise at stages in the life cycle at which crossing over and bar reversion do not normally occur, and, in the third case, there is definite evidence that crossing over did not occur. While it may still be supposed that unequal crossing over will sometimes give rise to section deficiencies, the evidence indicates that the three best-known examples of section deficiencies in Drosophila have not arisen in that way.

UNEQUAL CROSSING OVER AND THE EXACT NATURE OF SYNAPSIS

The data on crossing over have all indicated consistently that when two chromosomes cross over they do so at exactly corresponding levels. The case of bar is the first one in which any inequality of crossing-over levels has been detected; and we have seen in the preceding sections that an analysis of other possible instances of such an occurrence makes it probable that they must be explained in some other way. The case of bar is clearly quite exceptional. But it does serve to suggest that the exact correspondence of crossover levels, that is so constant, is not to be referred to a property common to all the genes. For unequal crossing over occurs in females that are homozygous for bar or for infrabar, and in such females these loci are alike in the two X chromosomes that cross over unequally. It is difficult to imagine how the chromosomes can pair so extremely exactly as they must do, unless in some way like genes come to lie side by side. But the present case indicates that this interpretation will have to be applied with some caution.

SUMMARY

1. Sixteen different kinds of changes at the bar locus are shown to occur exclusively, or nearly so, in eggs that undergo crossing over at or near the bar locus.

2. This result can be explained if it is supposed that such crossovers are unequal, so that one daughter chromosome gets two representatives of the bar locus while the other receives none.

3. Only one mutation in this locus has been shown to have occurred in the germ track of a male. This one gave rise (from bar) to a new and less extreme allelomorph called infrabar.

4. Infrabar does not appear to represent a quantitative change in the bar gene.

5. When, by unequal crossing over, bar and infrabar come to lie in the same chromosome, they maintain their separate identities, and may be recovered again.

6. In such double forms the two elements also maintain their sequence in the same linear series as the rest of the genes. It is thus possible to obtain bar-infrabar and also infrabar-bar. These two types look alike, but can be distinguished by their origin and by the usual tests for determining the sequence of genes.

7. Facet counts are given for all the possible combinations of the following members of the bar series: round, infrabar, bar, double-infrabar, bar-infrabar, double-bar.

8. Analysis of these data shows that two genes lying in the same chromosome are more effective on development than are the same two genes when they lie in different chromosomes.

9. A general survey makes it seem improbable that many mutations in other loci are to be explained as due to unequal crossing over.

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POLYPLOIDY IN *DROSOPHILA MELANOGASTER* WITH TWO ATTACHED X CHROMOSOMES

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INTRODUCTION

From a mosaic of *Drosophila melanogaster*, with a yellow female region in which, as the subsequent history showed, the two X chromosomes were attached to each other by their ends, a so-called "double-yellow" stock has been maintained. The two X's of the original fly both carried the differential for the recessive mutation, yellow body color, and since the X chromosomes remained attached, the stock consists of yellow females that are derived from eggs with attached X's, fertilized by Y sperm, and of males like their father that are derived from eggs with a Y chromosome, fertilized by X sperm. The two classes occur in equal numbers. Occasionally in the cultures an XX X female occurs, which develops from an XX egg fertilized by X sperm. She is gray in body color if her father has been a wild-type fly, and has characteristic irregularities of form and of eyes and wings by which 3X flies with two sets of autosomes are recog-

TABLE 1

Offspring of double-yellow females.

P1		F1				
Number	Mate of $\widehat{yy}$ ♀	$\widehat{XX}$ Y $\widehat{yy}$ ♀♀	$\widehat{XX}$ X Super-females	Patroclinous ♂♂	XX Gray ♀♀ one detached X from mother	Yellow ♂♂ detached X from mother
850bb	$w^e f$	*n*	0	*n*	..	1
856oa	Wild-type	71	0	80	..	1
770	$w^e c_t f$	*n*	4	*n*	..	..
813	Wild-type	84	0	71	0	0
850	" "	78	1	71	0	0
851	" "	21	0	14	0	0
852	*y w*	59	0	86	0	0
853	*y w*	35	0	56	0	0
854	X-ple	74	4	63	0	0
855	X-ple	11	0	8	0	0
856	$y\ b_r\ w^e$	73	0	68	0	0
75a	Mass, $w^e\ s_n$	75	55	60+	0	0
75b	Mass, $w^e\ s_n$	26	23	32	1?	0
15	Mass, $c_v\ c_t f$	138+	35(+?)	55+	1 or 2	0
1500	$c_v\ c_t f$	22	0	..	1	1(+?)
260	Mass, $c_v\ c_t f$	71	33	36	0	0
260a	Wild-type	42	..	35	..	..
260b	" "	36	..	39	..	..
771a	" "	98	15	125	0	0
771b	" "	66	4	56	0	0
771c	" "	148	7	125	0	0
771d	" "	105	5	100	0	0
32a, b, c, d, e	" "	536	5	556	0	0
54a, b, c, d, e	" "	485	3	543	0	0
144	" "	*n*	..	*n*	..	2
18	" "	*n*	..	*n*	..	2
18a	" "	*n*	0	*n*	0	0
18b	" "	*n*	0	*n*	0	0
18t	*f B*	*n*	0	*n*	$0\frac{B}{+}$	0
19	$w^e\ c_t\ vf$	24±	..	21	3	3
19b	"	*n*	1	*n*	0	0
19c	"	*n*	1	*n*	0	0
19m	*f B*	139	0	23	$2\frac{B}{+}$	0
184	*B*	14	0	62	$11\frac{B}{+}$	14
7-24*	w^a	4419	15	4257	9	4

* Eighteen generations of cultures which produced also 9 patroclinous apricot ♀♀.

nized. There is a fourth possible individual, one that would arise if an egg with a Y chromosome were fertilized by Y sperm; but a fly so constituted has not appeared and presumably dies. The number of 3X females varies from none to a few in a generation (see table 1); but in some cultures (75a, mass culture, and others) the number of 3X females is equal to that of other classes.

FEMALES WITH ATTACHED X'S

The genetic results in regard to sex-linked characters are those that are to be expected, according to the known behavior of chromosomes, if two like X chromosomes are attached, and sections of metaphase plates have also shown that the X's are present, joined at one end (cf. figure 1b). More details will be added to some of the results already reported (L. V. MORGAN 1922).

The autosomes were tested by three short experiments to find whether the race was normal for autosomal crossing over. The characters black, purple, vestigial were used for the second chromosome. A double-yellow female was crossed to a black, purple, vestigial male, and the yellow daughters, heterozygous for these II-chromosome recessive characters, were backcrossed to the recessive males. The second chromosome proved to be normal for crossing over as far as the test went (table 2). In a total of 361 flies, the percentage of recombination of black and purple was 6.4 where 6.0 was expected, and the percentage for the loci of purple and vestigial was 12.5, which was expected.

TABLE 2

Crossing over in autosomes II and III in double-yellow females.

CHROMOSOME	LOCI	PERCENT EXPECTED	PERCENT FOUND	NUMBER OF FLIES
II	b-p_r	6.0	6.4	361
	p_r-v_g	12.5	12.5	"
III	p-s_s	8–14	12.6	819
	s_s-e^s	9–15	14.1	"
	e^s-r_o	18–22	14.1	"
	s_t-s_r	14–20	23.6(♂♂only, 22.6)	1761
	s_r-e^s	8.7	8.2	"
	e^s-r_o	18–22	18.0	"
	r_o-c_a	9.6	9.9	"

The third chromosome was similarly tested with two different stocks. Accurate data on crossing over in the third chromosome show differences in behavior in the region including the middle of the chromosome, and

without a control the expectation for a given experiment can be known only within certain limits. With peach, spineless, sooty, rough, in a total of 819 flies, the percentage of recombination of peach and spineless was 12.6, where the expectation varies from 8 to 14, the percentage for spineless and sooty was 14.1 and the expectation is 9 to 15. The expectation for sooty and rough is 18 to 22, and the much lower figure 14.1 was obtained.

In a second test with scarlet, stripe, sooty, rough, claret, involving 1761 flies, the percentage for sooty and rough came up to 18.0, nearer to expectation but again low; this was partly offset by a percentage (in the same experiment) higher than expectation for scarlet and stripe, for which 23.6 was found and 14 to 20 is expected. 23.6 was based on counts of all the flies, but since the females were all yellow, and since stripe does not always show as clearly in yellow flies as in wild-type flies, the percentage for the scarlet-stripe section was calculated also from counts of males only; 22.6 was then obtained. For the other two sections the percentages were close to expectation; for stripe and sooty, 8.2 was found and 8.7 expected; for rough and claret, 9.9 found and 9.6 expected. On the whole, it may be concluded that crossing over in the third chromosome, as in the second, is normal in flies with attached X chromosomes. Crossing over in the X chromosome could not be observed because the two attached X's were alike and because the 3X flies were sterile.

PATROCLINOUS FEMALES

An exceptional kind of female, one homozygous for the sex-linked characters of the father, has been found rarely (about fourteen times) among the offspring of double-yellow females. They come presumably from Y eggs. One of these flies had wings which were cut out irregularly on the inner margin, a disturbance often found with certain unusual relations among the chromosomes. The fly died, and four other patroclinous females died or failed to breed even when transferred to fresh food with several mates.

Three other patroclinous females mated to males unlike themselves gave, in F_1, males like themselves and heterozygous daughters, and had, therefore, separate X chromosomes. One certainly, and perhaps two, produced a non-disjunctional daughter and a non-disjunctional son, showing the presence of a Y chromosome.

Another patroclinous female, which was apricot, mated to forked bar, gave no forked bar sons, but as many apricot daughters as there were sons or daughters of the regular classes. Four of the apricot daughters

were tested, but they gave no exceptional offspring. Non-virginity might explain the classes but not the ratios obtained in F_1.

BREAKING APART OF THE ATTACHED X'S

Occasionally the two X chromosomes of a double-yellow fly become detached, and an egg with a single yellow chromosome gives rise to a yellow male or to a wild-type female with normal body color if the father was not yellow, or to a not-gray heterozygous bar female if the father was bar. Flies with the detached yellow-bearing chromosome are often found singly as though the break between the chromosomes had not occurred early in the germ track; but there are also a few records of more than one fly with a detached chromosome from a pair mating. No. 19 (table 1) gave as many as six, three yellow males and three wild-type females which produced yellow sons, proving that they had received a single yellow-bearing chromosome. Two of the double-yellow sisters of the six flies with a detached chromosome were mated separately to brothers (19b and 19c) and none of the offspring had a single yellow-bearing chromosome, but a very nearly related double-yellow female (19m), outcrossed, gave two gray (heterozygous bar) females which proved each to have a single yellow chromosome. An unrelated culture, No. 18, gave at least two yellow males, and two pairs of F_1's (18a and 18b) and one double-yellow F_1 female (18t) outcrossed gave no more "breaks."

The largest number of "breaks" recorded from one pair of flies is in culture No. 184, a double-yellow female mated to a bar male, a pair from a stock that was giving a high ratio of males to females. The number of yellow males in F_1 was equal to the number of double-yellow females, and the number of F_1 heterozygous bar females with wild-type body color was nearly as large. Two of the females were tested and proved to be heterozygous for yellow and for bar, giving yellow sons and bar sons. No other cultures of the stock (which was being watched for other purposes) showed similar ratios, although there were other kinds of exceptional individuals, as will be described later.

The data do not indicate that the breaking apart of the chromosomes depends on an inherited condition in the attached-X females.

In order to find out whether the break between the chromosomes takes place at the original point of attachment, a number of the detached chromosomes were tested. If the chromosomes broke apart at a point other than that between the original chromosomes, one resulting chromosome would have a deficiency at one end and the other a corresponding duplication, presumably at the like end. Since it was not at the time

known by which end the chromosomes are attached, it was desirable to test both ends. The locus for yellow is very near the "left" end of the chromosome, and if the detached chromosome had the differential for yellow, there should not be a deficiency except possibly a very short one to the left of yellow. A duplication of the yellow locus could not be detected by the presence or absence of the yellow color because both chromosomes carry the differential for yellow. If there were a duplication as far as the locus for the dominant mutation, abnormal (3 units to the right of yellow), a female heterozygous for the detached chromosome and for abnormal would probably not show abnormal, because the character is incompletely dominant, and at the locus for abnormal, the fly would have two wild-type differentials and one for abnormal. The right end of the chromosome could not be easily tested, especially beyond the locus for bar. It was thought that in a female heterozygous for the detached chromosome and for forked and bar, a normal heterozygous bar eye and a not-forked condition would be proof that there was no duplication in that individual in the detached chromosome at bar, or deficiency at bar, or deficiency at the near locus of the recessive mutation, forked. The test is, however, not very useful, because a fly with a deficiency or a duplication for a distance much shorter than from the end of the chromosome to and including bar would probably (judging from known cases) have wing disturbances and lessened viability, and what the behavior of bar would be is not certain. The test with forked bar is therefore useful only to show whether flies with the detached chromosome give normal offspring of the classes and in the ratios expected.

The tested males and females came from various cultures in which the fathers had different sex-linked characters other than yellow.

Two yellow males were double-mated, each to a forked bar and to an abnormal female. The first matings in both cases gave in F_1 regular heterozygous bar females, not-forked, and the second matings gave F_1 females that were "abnormal." One of the males was also backcrossed to a heterozygous bar daughter and gave regular yellow females and the other expected classes. The detached yellow-bearing chromosomes were in every way normal. Eleven other yellow males showed normal behavior for the loci for yellow, abnormal (when the mating succeeded), forked and bar, and showed no somatic disturbances. Six yellow males failed to breed.

No more males were tested, since the chances of picking up a chromosome with any disturbance would be greater in a female, because a male zygote with a deficiency or duplication would probably die, but a female

might survive, owing to the presence of the other X chromosome, which is normal. Of females with a detached X chromosome received from a double-yellow mother, which were tested, fifty-nine gave yellow sons and other expected classes of males and females in an estimated 1:1 sex ratio. It was not determined whether or how often the maternal Y chromosome passed into the egg with the detached X chromosome. Fourteen not-yellow daughters of double-yellow females failed to breed.

In all, 72 tested flies showed no unusual condition at the loci for yellow, forked and bar, and those that were tested for the locus abnormal were regular. From examination of samples of the F_1 generation and from genetic tests of a few of the F_1's, it appears that the tested flies behaved as do normal flies which have a single (i.e., unattached) X chromosome carrying the differential for yellow.

Eight other females found at different times among offspring of double-yellow females have shown various types of unusual behavior, not, however, at least in some of them, concerned with the breaking apart of the attached chromosomes.

TRIPLOID FEMALE

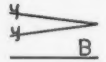
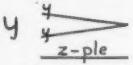
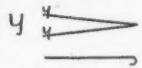
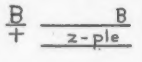
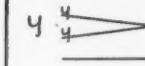
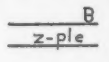
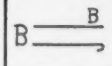
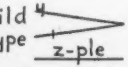
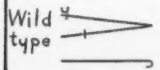
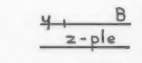
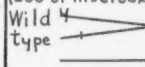
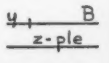
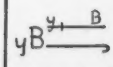
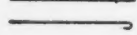
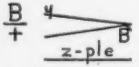
One of the flies which did not give expected genetic results was a large heterozygous-bar female (No. 182) of wild-type but slightly yellowish color, found by T. H. MORGAN in a culture of double-yellow females and bar males. She was mated to a male with the recessive characters scute, echinus, crossveinless, vermilion, garnet and forked, a combination called "z-ple."

Among the offspring (table 3), there were, besides males and females, a number of intersexes.[1] Forty of the offspring were mated; the females proved to be of three kinds: first, those that gave 100 percent non-disjunction in the next generation like the double-yellow race (though not all of these were yellow); second, those whose two sex chromosomes were separate X's, as in common females; and third, those that repeated the behavior of the mother, and gave again three kinds of females, and males and intersexes. The race was carried on by mating females that gave intersexes; these females may often be recognized by the large size and

[1] For descriptions of the characteristics of intersexes see BRIDGES (1921, 1922). Intersexes are most easily recognized when the abdomen is like that of a female, because sex combs are always present. But the abdomen is sometimes almost like that of a male. Classification is then based on other variable conditions, such as a broadness of the head and thorax, or disturbances of the wings which are sometimes cut out on the inner margin, or compressed laterally, sometimes with incomplete venation. When the eye is heterozygous bar it is not bean-shaped, as it often appears in females, but it is narrower and more truly bar-shaped.

TABLE 3

F_1 offspring of P_1, heterozygous bar *3n ♀ × "z-ple" ♂. Percentages of classes of F_1 offspring from 3n females with two attached X chromosomes and one free X chromosome, mated to 2n males.*

P_1	F_1					
182	Females			Intersexes		Males
	$\widehat{XXX}3A$	$\widehat{XX}Y2A$	$XX2A$	$\widehat{XX}Y3A$	$XX3A$	$XY2A$
						
	1 (tested)	6 (5 tested)	8 (7 tested)	1 (mated, failed) (2♂♂ or intersexes?)	B or	16 (4 tested)
by						
"z-ple" sc ec cv vg f	3 (tested)	9 (3 tested)	1 (tested)	2 (1 mated, failed)	20 (9 mated, failed)	2 (1 tested)
						
	1 (tested)					
	1 (failed to breed)					
Total 1474	9.0%	30.1%	18.4%	4.8%	20.7%	17.0%

granular surface of the eyes, and, when bar is present, by a very broad type of bar.

Another line which gave apparently parallel results was discovered by STURTEVANT. It was derived from the double-yellow stock through a patroclinous female with a new origination of the attached-X condition. It was evident from the behavior of both lines that three X chromosomes were present in the mothers of intersexes, but no part of the X chromosome seemed to be primarily responsible for the results that were found. STURTEVANT concluded that they were probably to be accounted for by the presence of three instead of two sets of autosomes, as in the 3*n* individuals described by BRIDGES (1922). The hypothesis was confirmed by cytological evidence, when preparations of the ovaries of a fly (from the stock from No. 182), which was selected as being of the class of mothers of intersexes, showed three complete sets of chromosomes (figure 1a).

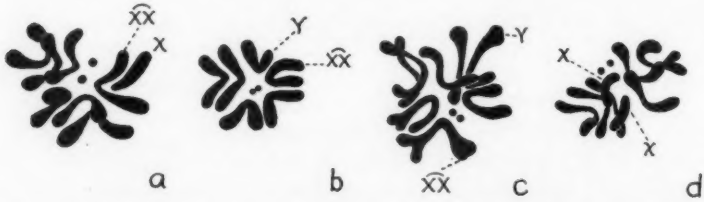


FIGURE 1.—Chromosome groups of: *a*. 3*n* female with two attached X's, one free X, and three sets of autosomes. *b*. 2*n* non-disjunctional female with two attached X's and a Y, descendant of a 3*n* female. *c*, *d*. Intersexes with three sets of autosomes, offspring of 3*n* females, *c*, with two attached X's and a Y, *d*, with two free X's.

The three sex chromosomes consist of two attached X chromosomes (as found in the double-yellow stock) and of one free X chromosome. The kinds of individuals that are expected from such a female mated to a 2*n* male are shown in table 4. BRIDGES (1922) has discovered that from 3*n* females (with separate X's) no flies survive which have three of one of the two large autosomes and two of the other large autosome; that is, autosomes II and III are either both diploid or both triploid. The behavior of autosome IV is different, and in the present account, 3*n* stands for 3 X, 3 II, 3 III, irrespective of the number of IV's that are present. The eggs are of four kinds: those with two attached X chromosomes and two sets or one set of autosomes, and those with a single free X chromosome and two sets or one set of autosomes (table 4). The four kinds of eggs fertilized by X sperm (with one set of autosomes) give the flies whose sex chromosomes are diagrammatically represented in the upper row in

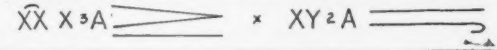
table 4, and the four kinds of eggs fertilized by Y sperm give the combinations represented in the lower row.

Of the offspring of No. 182 four wild-type females* and one female with a broad type of heterozygous bar eye (table 3, column 2) were mated to *2n* males, and gave in the next generation, males, females and intersexes, which proved that the F_1 females, like their mother, were *3n* flies.

Five of the six F_1 yellow females (table 3, column 3) were tested and gave in F_2 yellow females and males like their father, that is, 100 percent non-disjunction, the result of having attached X chromosomes (both carrying yellow). They evidently came from eggs which received the

TABLE 4

Diagram of sex chromosomes of the different classes of F_1 offspring of a 3n female with two attached X chromosomes and one free X chromosome, mated to a 2n male.



sperm \ eggs	X̂X 2A	X̂XA	X 2A	XA
XA	3n ♀	super ♀ (rare, sterile)	intersex	♀
YA	intersex	♀ non-disjunctional	super ♂ (rare, sterile)	♂

attached X chromosomes of the mother, fertilized by Y sperm. Figure 1b is drawn from a division plate of a descendant of such a female from another culture, and the sex chromosomes are shown to be two attached X's and a Y. In cytology and genetic behavior they are like females of the double-yellow stock. Out of nine other wild-type females, three were tested (table 3, column 3) and gave 100 percent non-disjunction. All of their sons were like their mates, but their daughters were of two kinds, wild-type and yellow,—crossing over between the X chromosomes was taking place, as will be described later.

Eight other heterozygous bar females (table 3, column 4) had two separate X's, since seven of them gave bar sons, z-ple sons and sons of crossover classes between bar and z-ple, and one gave yellow bar sons, z-ple sons and crossovers. They behaved genetically like common females,

* The first fly in column 2 has been wrongly named y (=yellow) in table 3.

and undoubtedly came from eggs with the free X chromosome carrying bar (in one case also yellow) fertilized by X sperm which carried the differentials for the characters of z-ple.

The constitution of the intersexes in regard to sex chromosomes can be inferred from the phenotypically similar females, since the intersexes differ from females only in the number of autosomes. Like the $2n$ females they are of two kinds, one with two attached X's from their mother and a Y chromosome from their father (table 3, column 5), and the other kind (table 3, column 6) with one X from each parent. Preparations of metaphase plates of intersexes from No. 182 were not made, but from other suitable matings, the two kinds of intersexes could be inferred from the phenotypes. Figures 1c and 1d are drawn from chromosome groups of intersexes from two different cultures. In both, the autosomes are triploid. Figure 1c is from a wild-type intersex whose $3n$ mother had differentials for forked and bar in the free chromosome and no mutant differential in the attached X's. She was mated to a wild-type male and the F_1 intersexes that were bar showed that they were of the class that received the free bar chromosome from their mother and had therefore two separate chromosomes (unless they were of the very small class of crossovers). The not-bar intersex, whose chromosomes were drawn, should have attached X's from the mother and a Y chromosome from the father, the kinds of chromosomes which the figure shows. Figure 1d is drawn from a chromosome group of a wild-type intersex of different parentage. The $3n$ mother had a differential for bar in the attached X's and no mutant differential in the free X chromosome. She was mated to a wild-type male. Her wild-type (non-crossover) intersex offspring should be those that had a single X chromosome from their mother and an X from their father; two separate X's are found in the sections. Eleven F_1 intersexes of No. 182 were mated as males or as females, according to the type of abdomen, and all failed, showing the usual sterility of intersexes. Five males (table 3, column 7) were mated to different kinds of females and proved to be fertile and gave expected classes of offspring.

Many generations of $3n$ flies have been bred, and it has been found that, although the number of offspring of No. 182 was not large, all of the non-crossover and crossover classes which are derived from a $3n$ female in which two of the X chromosomes are attached were represented, except the rare 3X 2A "super-females" and X 3A "super-males."

That the $3n$ females were such has been shown by the counts of chromosomes in the metaphase plates and also by the regular occurrence of $3n$ females and of intersexes in successive generations. The X chromo-

somes were marked, and their behavior, which was similar to that of the X chromosomes of the first culture, furnishes specific genetic evidence that there were three of them in the *3n* females. In some cultures the second chromosomes also were marked and the results have added genetic proof of the presence of three autosomes in the *3n* females and intersexes. A *3n* female was mated to a male which carried in one second chromosome the differentials for curly and black, and also for a factor which prevents crossing over in the region between them; curly is a dominant character and black is recessive. In F_1, the curly *3n* flies had two wild-type second chromosomes from their mother and one with curly and black from their father. *2n* offspring of such an F_1 female, which was mated to a black male, were either curly and black or not-curly and not-black. Some of the female offspring with three sets of autosomes were curly and not-black; they had one chromosome with black from their father, one with curly and black from their mother, and one wild-type chromosome with neither of the mutant differentials, from their mother. They bred as *3n* females and, mated to black, gave again in F_3 some curly not-black females and intersexes, classes that could occur only as the result of the presence of three sets of autosomes. The line is continued by repeating the last kind of mating. The interference with crossing over makes it certain that a curly not-black offspring of a black father has developed from an egg which had two second chromosomes, one curly because the fly is curly, and one wild-type because the fly is not-black.

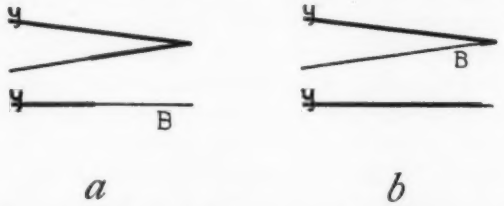
The percents of different classes of F_1 offspring of *3n* females have been calculated from records of 17 cultures which had been followed primarily to ascertain the kinds of flies in F_1 rather than the exact number of each kind. In a total of 996 flies, 8 percent were *3n* females, 30.0 percent attached-X females, 17.5 percent separate-X females, 4.6 percent attached-X intersexes, 22.2 percent separate-X intersexes and 17.5 percent males.

In order to check the numerical results just quoted, more complete counts were made of 9 cultures of which the doubtful females were tested by breeding. The *3n* mothers had differentials for forked and bar in the free X chromosome, and the normal allelomorphs of both in the attached X chromosomes; the second chromosomes were one curly black, one black and one wild-type; they were mated to forked bar black males. The offspring that could be classified without testing were: not-bar females and intersexes which had attached X's; forked bar females and intersexes which had separate X's; not-forked, heterozygous bar, curly, not-black females which were *3n* females; and males. The flies of one remaining phenotype, which was not-forked heterozygous bar, were either *3n*

females, crossover females, or intersexes. The last two might have attached X's or separate X's and the females were bred and rated by their offspring. Nearly all the tests succeeded and the counts were the basis of the following percentages: the total number of flies was 478, of which there were 10.2 percent of $3n$ females, 30.7 percent of attached-X females, 20.2 percent of separate-X females, 5.2 percent of attached-X intersexes, 17.5 percent of separate-X intersexes and 15.8 percent of males. The results are fairly in accord with the first set of figures and the disparity between them relates as much to percentages of males, females and intersexes as to the percentages of different kinds of females and of different kinds of intersexes (the latter being not accurate in either set of counts, since the crossovers could not be tested). Among the flies of the first group of cultures there were 55.5 percent of females, 26.8 percent of intersexes and 17.5 percent of males. Those of the second group were 61.1 percent of females, 22.7 percent of intersexes and 15.8 percent of males. The percentages in table 3 have been calculated from both groups together.

CROSSING OVER IN TRIPLOID FEMALES

With the introduction into the double-yellow stock of the third complete set of chromosomes, crossing over of an attached X chromosome could, for the first time in the history of the stock, be detected, since the third



a *b*

FIGURE 2.—Two possible crossovers between two attached chromosomes, both carrying yellow, and a free chromosome carrying bar.

X did not carry yellow, and the extra autosomes made possible the breeding of females with three X's. The F_1 males of No. 182 were of two classes, the non-crossover class bar, like their grandfather, and one kind of crossover, namely, yellow bar, but not the usual complementary class, which, in this case, would be wild-type. The explanation is found in the fact that the free chromosome crosses over with one of the two attached chromosomes. (In No. 182, a chromosome carrying bar crossed over with an attached yellow-bearing chromosome, figure 2a.) One piece of the free chromosome becomes part of an attached chromosome, and it goes

therefore to a female fly and never to a male as in ordinary crossing over. Eggs with crossover chromosomes, when fertilized by Y sperm, become, if they receive the free chromosome, males or super-males, and in the complementary class, receiving attached chromosomes, they become non-disjunctional females or intersexes, according to the number of autosomes in the egg. Some of the non-disjunctional F_1 females of No. 182 were yellow (showing no crossing over) and others were wild-type, and these have been proved by breeding to have had a yellow-bearing chromosome attached to a wild-type chromosome. It is therefore the left end (wild-type) of the bar chromosome that crossed over to the attached pair, and the left yellow-bearing end of the attached pair was shown by the presence of yellow bar F_1 males to have crossed over to the free chromosome (cf. crossover classes in table 3, columns 3, 7). Since these two complementary classes, namely, yellow bar males and non-disjunctional females heterozygous for yellow, prove that the left end of the attached chromosome crosses over to the free chromosome, it follows that the joined chromosomes are attached to each other by their right ends.

The fact of the attachment by the right end can be proved as well by the examination of the other complementary classes in F_1. Eggs with the single crossover chromosome, shown in figure 2a, fertilized by X sperm, become females or intersexes with separate X's (according to the number of sets of autosomes in the egg) and eggs with the attached crossover chromosomes fertilized by X sperm are the complementary class and become (according to the number of autosomes) 3X 2A females (which usually die), or 3X 3A females. Out of eight tested heterozygous bar (not-3*n*) F_1 females (table 3, column 4), seven proved to have received the free bar-bearing chromosome from the mother, and one had received a crossover yellow bar chromosome (the same combination that was found in the crossover males). Again, the left end of the free chromosome carrying bar was found to have crossed over in three of the tested wild-type 3*n* females (table 3, column 2), because in them the attached chromosomes were found by breeding to be one yellow-bearing and one wild-type chromosome.

In the individuals with a crossover chromosome, so far described, crossing over had taken place in the region between the loci for yellow and bar. In one other 3*n* female (which was heterozygous bar, table 3, column 2) crossing over had occurred to the right of bar as proved by the constitution of her attached X's. They were found by breeding to be a yellow-

bearing chromosome attached to one carrying the differential for bar but not for yellow (cf. figure 2b).

In all the crossover chromosomes, at whatever level they crossed over, whether in eggs that gave rise to males, to females with separate X's, to non-disjunctional females with attached X's, or to *3n* females, it was the left end of the free X that had interchanged with the left end of one of the attached X's. Abundant confirmation was found in subsequent cultures of males and tested females, and the records of the F_1 males of two cultures are given as further examples.

In the first example the tested *3n* female had the differential for scute (a character at the left end) in one attached chromosome, and the differential for vermilion in the middle of the other attached chromosome, and had in the free chromosome the differentials for forked and bar (whose loci are toward the right end). Her sons were 5 non-crossover forked bar, one scute forked bar, and one vermilion forked bar. A female, with forked bar in one attached chromosome and scute, echinus, crossveinless, cut_6, $garnet_2$ in the other, had a free chromosome with differentials for yellow, and for hairy-wing, loci at the left end. The F_1 males were 6 non-crossover yellow hairy-wing, and 1 scute echinus, 1 wild-type, and 1 scute, echinus, crossveinless, cut_6.

CROSSING OVER BETWEEN THE ATTACHED CHROMOSOMES

It has been shown that an attached X chromosome of a *3n* female may cross over with the free X chromosome; differentials for sex-linked characters can therefore be introduced into the attached pair of chromosomes. In this way a *3n* female was obtained in which one of the attached chromosomes carried the differential for yellow and the other chromosome the differentials for scute, echinus, crossveinless, vermilion, garnet and forked, a combination already referred to as "z-ple." A wild-type *2n* non-disjunctional daughter from an egg with attached chromosomes of this constitution gave in F_1, wild-type daughters, yellow daughters and daughters showing various combinations of the mutant characters. Crossing over was taking place, which involved the two attached X chromosomes carried by the females of the line. Some points in regard to crossing over of attached chromosomes were observed by breeding the wild-type females for a few generations.

RECESSIVES SHOWING MUTANT CHARACTERS DETERMINED BY DIFFERENTIALS IN THE LEFT END OF THE CHROMOSOME

The findings recorded in table 5 show that a wild-type fly with recessive characters in two attached chromosomes produces some daughters that

are recessives. It is evident that interchange between two chromosomes attached to each other (e.g., those represented by AB, figure 3a) would not change the phenotype in F_1. Recessive individuals could be produced from two attached chromosomes only if the interchange took place after division of the chromosomes and then between the unlike not-joined strands of the double pair (e.g., between A and B′), resulting in two attached chromosomes, such, for example, as those represented by AB in figure 3b. (A′ and B′ are reversed in position as compared with A′ and B′ in figure 3a.) Since recessives in fact occur, crossing over must, sometimes at any rate, take place in the four-strand stage between the strands just named.[2] If crossing over took place as represented in figure 3b, the fly from an egg receiving the chromosomes AB would show those characters of z-ple that had become homozygous, namely, those determined by differentials in the left end of the chromosome, and would be scute, echinus, crossveinless. If at the same time crossing over took place between the attached strands to the left of a marked locus (such as *f*) the distribution of the differentials would be changed, but not the phenotype (as in AB, figure 3c). The second crossing over could be detected in the next generation.

RECESSIVES SHOWING MUTANT CHARACTERS DETERMINED BY DIFFERENTIALS IN THE RIGHT END AND MIDDLE OF THE CHROMOSOME

The phenotypes of the usual complementary classes when single chromosomes are in question occur in F_1 from attached X's (table 5), but both classes cannot be the result of a single crossing over of attached chromosomes. For instance, the chromosomes B′A′, which are complementary to AB (figure 3b), would not give rise to a recessive showing vermilion, garnet, forked, the supplementary z-ple characters in the right end of the chromosome B′; because this is attached to a chromosome with the wild-type allelomorphs. Those characters could appear in a fly if A and B′ crossed as before only in case a simultaneous crossing over occurred between B and A′ (figure 3d), such that A′ would receive from B a piece including the loci for the right-end characters, vermilion, garnet and forked in the example. The second crossing over must take place at a level to the right of the locus of the last character (forked), and as the fly is not yellow, it must be a single crossing over, as represented between B and A′, figure 3d.

[2] The same conclusion has been reached by ANDERSON and STURTEVANT from similar data not yet published, and by BRIDGES from a study of 3*n* females with separate X's.

The resulting chromosomes A′B′ would give the vermilion, garnet, forked phenotype. Because of the attachment of the right ends of the chromosomes, recessives involving the right-end characters are in the class of phenotypically double crossovers. They occur less frequently than scute, echinus, crossveinless flies having the characters determined by the left end. This is expressed in table 5 by the inequality in numbers of the classes showing supplementary blocks of recessive characters, since the counts are made from F_1's of females of like constitution. The smaller classes are probably heterogeneous, as will presently appear, though not in the way described as possible for the larger classes. The true complementary classes, when two attached chromosomes are involved, of which

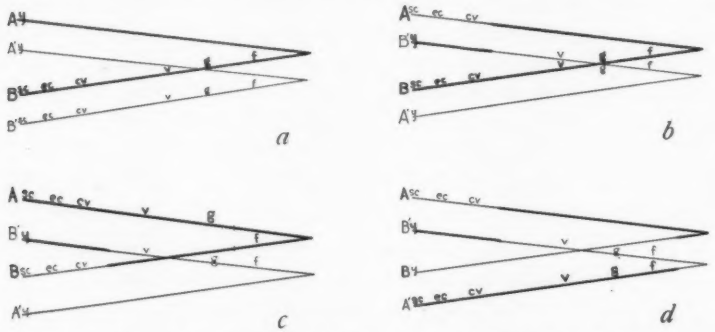


FIGURE 3.—Diagram of: *a*. Two attached chromosomes after splitting. *b*. Single crossing over near the left end between two unlike not-joined strands. An egg with chromosomes AB would produce a scute, echinus, crossveinless recessive. *c*. The same crossing over with a second crossing over toward the right between joined chromosomes. *d*. The same crossing over, with a second crossing over to the right between the other pair of not-joined chromosomes. A yellow fly with chromosomes A′B′ of figure *b* is the real complement of a recessive with chromosomes AB of figure *b*.

only one is marked, are recessives showing characters determined by the left end, and wild-type flies that have lost those characters and are heterozygous for the supplementary characters; the latter class can be recognized only by their F_1 offspring. On account of the presence of yellow in the second X chromosome, the class complementary to the recessives with characters of the left end in the flies recorded in table 5 is among the yellow flies (cf. figure 3b).

The phenotypes that are like double crossovers of single chromosomes (e.g., vermilion, garnet) might come about in two ways, either by a double crossing over between two unlike not-attached strands (figure 4a), or by two single crossings over at different levels that overlap, one between

TABLE 5

F_1 females from wild-type mothers with attached X chromosomes. P_1 11c to 101d, $\left(\frac{v}{s_c\ e_c\ c_v\ v\ g\ f}\right)$ ♀♀. P_1, 49 m (bottom line), ♀ with same mutant characters differently distributed. P_1, 11d to 49n, ♀♀ lacking some of the characters of the right end.

NUMBER	WILD-TYPE	*y*	*s*$_g$	*e*$_a$ *c*$_u$ *v* *g* *f*	*s*$_a$ *e*$_a$	*c*$_u$ *v* *g* *f* *v*	*s*$_g$ *e*$_a$ *c*$_u$	*v* *g* *f*	*s*$_a$ *e*$_a$ *c*$_u$ *v*	*g* *f*	*s*$_a$ *e*$_a$ *c*$_u$ *v* *g*	*f*	*s*$_c$ *e*$_r$ *c*$_u$ *v* *g* *f*	*e*$_c$	*c*$_u$	*v*	*g*	*e*$_r$ *c*$_u$	*c*$_u$ *v*	*v* *g*	*e*$_r$ *c*$_u$ *v*	*c*$_u$ *v* *g*	*e*$_c$ *c*$_u$ *v* *g*	
11c	5	3	..	..	..	..	3	1	..	..	2	..	..	..	..	..	..	..	..	..	..	1	..	..	
11e	42	16	..	..	2	..	1	..	..	2	4	..	..	..	..	1	4	1	..	1	1	..	1	1 *ygf*, 1 *yg*
11f	10	5	..	..	2	..	..	..	1	2	1	..	1	..	..	2	..	..	..	..	..	..	..	
11g	16	3	..	1	..	..	2	1	..	..	3	..	..	..	..	..	1	..	..	.. *	..	1	..	
12a	11	4	1	..	..	..	1	..	..	..	1	3	..	..	..	..	..	..	..	..	..	..	..	
17d	42	12	2	1	3	..	6	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2 $s_c\ e_c\ c_r\ g$ (f in F_2)
29c	29	12	1	1	1	..	1	..	..	..	..	2	..	1	..	1	..	..	..	1	..	..	..	
29d	17	6	..		..	..	1	..	1	..	..	1	..	1	..	..	..	1	..	1	..	..	..	
29e	26	15	..		1	..	2	..	..	1	2	1?	..	..	..	..	1	..	2	3	..	2	1	
30b	25	9	2		3	1	2	2	..	1	1	..	..	..	..	..	..	..	..	1	..	1	..	1 *y v g f*
30f	72	25	..		6	..	11	..	2	1	..	..	1	..	2	3	..	..	..	1	..	1	1	1 $s_c\ e_c\ g$, 1 $s_c\ e_c\ vg\ f$, 1 e_c ?
30g	38	12	..	2	4	..	5	2	..	1	..	1	..	..	..	..	..	1	1	4	1	..	..	1 *ygf*
31d	52	23	2		..	1	4	4	..	..	1	1	..	..	..	1	..	..	..	1	..	..	1	
32a	37	6	1		2	1		..	10	..	..	1	..	..	2	..	..	1	..	..	..	..	..	1 $s_c\ e_c\ c_v\ f$
49a	31	5	2		1	..	4	1	1	1	3	..	..	..	..	..	..	..	..	1	..	1	..	
49j	59	14	..	1	4	..	6	3	2	1	2	..	..	..	..	..	..	..	2	1	..	..	1	1 $s_c\ e_c\ c_v\ g$
49r	39	21	1		3	..	8	..	1	..	5	1	..	..	..	3	6	1	1	..	..	..	..	1 $s_c\ e_c\ vg$, 1 *ygf*, 1 $\frac{B^s}{+}$
44c	13	11	..	1	2	1	4	..	2	..	..	..	..	..	..	..	2	..	..	4	1	1	..	
55c	23	12	1		1	1	8	2	..	1	1	1	..	..	..	..	..	..	1	1	..	..	..	
101a	37	6	1		2	1	11	..	2	2	2	..	..	..	..	1	..	..	..	2	..	..	2	
101b	13	8	1		..	..	1	..	..	..	1	..	1	..	..	..	..	..	..	1	..	1	..	
101d	18	4	..		1	..	2	1	..	..	..	..	..	..	..	1	..	..	..	..	1	..	..	1 *yf*
Totals	655	232	15	6	38	6	83	17	26	13	29	12	3	3	4	13	14	5	7	23	5	8	7	
11d	56	12												8										
17f	38	8	4																					
49h	45	15	2		10									2										
49n	45	16	1		15									2										

49m: 43 wild-type, 6s_c, 3*y*, 4 *y* e_c c_v, 3 *y* e_c c_v *v*, 3 *y* e_c c_v *v* (g?), 2 s_c *g*, 1 s_c *gf*, 1 e_c c_r *v*, 3 c_v *v*, 1 c_v *vg*, 1*v*, 2*g*, 3*gf*.

* Derived from an egg with a detached X chromosome, fertilized by X sperm carrying bar.

the two members in each of the two pairs of unlike not-joined strands (figure 4b). The differentials would be differently distributed in the chromosomes in the two events, and the amount of crossing over that had taken place would be in the first case twice in one pair of chromosomes, and in the second case twice in two pairs, which, with detached chromosomes, would count for two flies. Recessives showing right-end characters (figure 3d, A′B′) are, as shown above, cases of double crossovers and might arise in either of the two ways. That both processes do in fact sometimes take place is shown by the occurrence of flies which are yellow and at the same time homozygous for one or more recessive characters carried by the other chromosome of the mother. For example, the yellow, garnet, forked

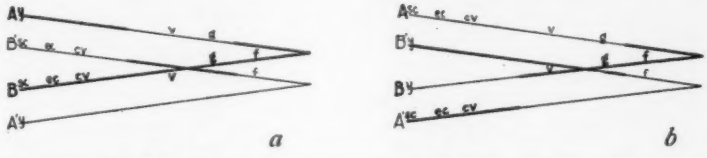


FIGURE 4.—Diagram of: *a*. Double crossing over between two unlike not-joined chromosomes. *b*. Single crossings over at different levels between the unlike chromosomes of both of the not-joined pairs. Resulting phenotypes with the chromosomes AB of *a* and of *b* are the same, but the distribution of differentials in the chromosomes is different.

fly whose mother was of the given constitution must have resulted from two simultaneous crossings over between two unlike not-joined pairs of strands, namely, one single crossing over and one double crossing over. Wild-type flies that have lost some of the mutant differentials of the middle or right end (as A′B′ in figures 4a and 4b) are of the class complementary to the double crossovers by either process; they can be recognized only by their F_1 offspring. For example, in table 5, P_1 females of cultures 49h or 49n are complementary to crossveinless, vermilion, garnet, forked flies.

REDISTRIBUTION OF DIFFERENTIALS

That a redistribution of differentials without change of phenotype and without loss of any mutant differential sometimes occurs is shown by cultures of which 49m (table 5) is an example. The redistribution might have come about either by crossing over between two unlike joined strands (figure 5a single, figure 5b double crossover) or by crossing over between two unlike strands not attached to each other, if at the same time an interchange took place at the same level between the other two unlike not-joined strands (figures 5c and d). The amount of crossing over would be twice as much in the latter case, but the constitution of the

F_1 flies (for example, those receiving the chromosomes AB of figure 5, a and c, or the double-crossover chromosomes AB of figures 5, b and d) would be the same and genetic tests would not show which had occurred.

The results from the breeding of the wild-type flies show that with the various possibilities of crossing over which produce the same phenotypes, the amount of crossing over can not be known without genetic tests of all of the F_1 flies and comparison of results from mothers that by their offspring are proved to have been of similar constitution.

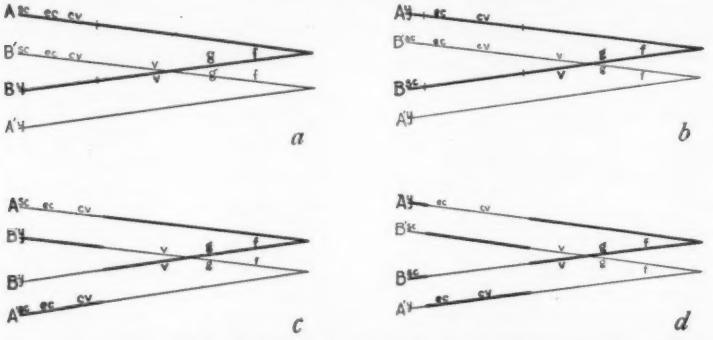


FIGURE 5.—Diagram to show redistribution of differentials in chromosomes AB. *a*, by single crossing over between two joined chromosomes, or *c*, same redistribution by single crossings over between unlike chromosomes of both of the not-joined pairs. *b*, and *d*. The same alternatives involving double crossings over.

CROSSING OVER AMONG THREE CHROMOSOMES

It has been shown that in $3n$ females crossing over takes place between an attached X and a free X, and that it takes place also between two attached X's; it sometimes occurs that a crossover chromosome is made up of parts of all three chromosomes; one example of this kind was a yellow, hairy-wing, broad, vermilion, garnet, forked, bar male which was

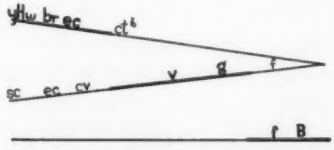


FIGURE 6.—Diagram of crossing over among three chromosomes.

probably also echinus (a character not always distinguishable when bar is present). The constitution of the X chromosomes of the mother is represented in figure 6, where the parts which made up the chromosome

received by the egg, from which the male developed, are represented by heavy lines. Crossing over in the attached chromosomes between the loci for crossveinless and cut, and crossing over with the free chromosome at a point between the loci for garnet and forked would result in a chromosome of the kind to produce the male observed.

RECURRENCE OF ADDITIONAL CHROMOSOMES

At two different times, a $2n$ female of a non-disjunctional line, whose parentage goes back to the first $3n$ fly, has produced, besides her regular non-disjunctional offspring, a fly that was a $3n$ female, as proved by breeding. Other exceptions found in three non-disjunctional cultures were recognized as intersexes by the presence of sex combs and more or less female abdomens. Two of the three mothers of these intersexes were attached-X daughters of a $3n$ female, and the third was a daughter of their $3n$ sister. The first was mated to apricot and produced the expected non-disjunctional daughters and apricot sons, a number of 3X 2A daughters and two or three apricot intersexes. The second was wild-type and was mated to an eosin, ruby, bar male, and produced besides the non-disjunctional daughters and sons, one wild-type intersex. The third was wild-type and mated to eosin miniature; she produced, besides females (wild-type and crossover) and eosin miniature males, one intersex that was miniature but had red and not eosin eyes. The apricot intersexes suggest a comparison with patroclinous females, if in the P_1 male non-disjunction of autosomes as well as of the X chromosome occurred at some division. Non-disjunction of maternal autosomes, or of paternal autosomes in Y sperm, would result in the phenotype of the wild-type intersex, but neither of the assumptions explains the miniature not-eosin intersexes.

It may be noted here that in the various non-disjunctional lines from $3n$ females the same kinds of exceptional flies have been found that were described as having occurred in the double-yellow stock (table 1). They were 3X 2A super-females, and males and females with a detached maternal chromosome, and two patroclinous females, one of which was shown to be non-disjunctional with separate chromosomes.

TETRAPLOID FEMALE

Still another instance of increase in the number of chromosomes was found in a daughter of a $3n$ female, which proved to be a $4n$ female; she behaved genetically as would be expected if she had four X chromosomes and four sets of autosomes. She had eyes so slightly bar that they appeared

to be normal except for a slight nick in the anterior edge. Her mother (23ea, table 6, column 1) carried in the attached chromosomes the differentials for yellow and the dominant character hairy-wing, and the differentials for forked and bar, and in the free chromosome the differential for miniature wing. The mother was mated to a miniature male and she produced flies of the usual classes of males and females and intersexes, and, in addition, the female with nicked eyes (28a). If this daughter (28a, table 26, column 2) were a 4*n* female she would be expected to have as sex chromosomes the attached pair like that of her mother (if no crossing over had occurred) and two free X chromosomes, both with the differential for miniature, if one came from her mother and one from her father. The two free X chromosomes would also both have the differential for miniature if she had received a double set of all of the paternal chromosomes.

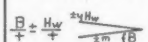
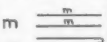
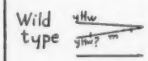
The 4*n* female was mated to a male which was wild-type in respect to sex-linked characters. Her offspring (table 6) were all (with one exception) either 3*n* females or intersexes; none of them was a 2*n* female or a male. The regular eggs of a 4*n* fly would be expected to have two X chromosomes and two sets of autosomes, and these fertilized by sperm with one set of autosomes would give only offspring with three sets of autosomes. The eggs that were fertilized by X sperm should be 3*n* females, those by Y sperm should be intersexes (2XY 3A).

Twenty-one of the F_1 females were tested, and proved to be 3*n* females. They were of two kinds, heterozygous bar and wild-type (the character hairy-wing will for the present be disregarded). If their mother (P_1) had the supposed combination of X chromosomes, the heterozygous bar F_1's (table 6, column 4, of the non-crossover class) should have in the attached chromosomes one differential for forked and bar. The daughters of these (F_2, table 7) that received the attached chromosomes should be non-disjunctional heterozygous bar females (or forked bar or wild-type by crossing over). These would give in F_3 (table 7) daughters like themselves, a few crossovers, and sons like their mates.

On the other hand, the wild-type F_1 females should have (except one rare kind of crossover) 3 separate X's (table 6, column 5), and they should produce some non-disjunctional miniature daughters, from eggs with two separate miniature-bearing chromosomes, fertilized by Y sperm (F_2, table 7, below the double line). These should give in F_3, if mated to males with a differential for bar, heterozygous bar daughters, miniature males, and a few non-disjunctional individuals, i.e., miniature females and males like the father.

TABLE 6

Parentage and F_1 offspring of a 4n female with two attached X chromosomes and two free X chromosomes. P_1, heterozygous hairy-wing heterozygous bar $\left(\frac{y\ H_w}{B}\ \frac{m}{m}\right)$ *4n ♀ × wild-type ♂.*

Parentage of P_1 ♀	P_1	F_1						
23ea	28a	Females			Intersexes			Males
$\widehat{XXX}$ 3A	$\widehat{XXXX}$ 4A	$\widehat{XXXX}$ 4A	$\widehat{XXX}$ 3A	XXX 3A	$\widehat{XXY}$ 3A	XXY 3A	$\widehat{XXXX}$ 3A	none
		slightly B		Wild type ± Hw / +		m / m 	slightly B	
by	by	1 (tested = 4n(?))	17 (9 tested = 3n		4	9	2 (failed to breed)	
XY2A	XY2A		Wild type 	15 (11 tested = 3n		Wild type + / B		
			1 (tested = 3n					

The records given in table 7 show that these expectations were realized. Tests of F_2 (or F_3) females from five different heterozygous bar F_1 females, showed that the F_1 heterozygous bar females had received attached chromosomes from their mother. (Miniature appeared in F_3 (or rarely in F_2) non-disjunctional females, when the attached chromosomes had received miniature by crossing over with a free chromosome in the $4n$ P_1 female.) Tests of F_2 miniature females, from three wild-type F_1 females, proved that these F_1's had received two free miniature chromosomes.

One of the not-bar F_1 females, 20q, had received attached chromosomes (as judged by the offspring) in which one crossing over had occurred with the free miniature-bearing chromosome at a level to the right of bar and (perhaps in the same generation) another crossing over with yellow hairy-wing (table 6, column 4). Non-disjunctional F_2 females were homozygous for yellow hairy-wing (44x and y) and heterozygous for miniature (see F_3).

Further evidence of the $4n$ condition is seen in the occurrence of F_1 intersexes that were miniature. The attached chromosomes of the mother carried bar, and these account for the bar intersexes (table 6, column 6). The father was wild-type and eggs that produced the intersexes homozygous for miniature (table 6, column 7) must have received two miniature-bearing chromosomes from their mother, and have been fertilized by Y sperm, that is, the miniature intersexes are accounted for if the mother had two free chromosomes (carrying miniature) in addition to the two attached chromosomes. The crossover class of intersexes with the two free chromosomes (table 6, column 7) should be wild-type; wild-type intersexes were found, but add nothing positive to the analysis. The genetic results prove the presence of two attached and two separate X chromosomes in the P_1 female, four X chromosomes in all; and the normal form and the fertility of the P_1 female and the classes in F_1 make probable without doubt the presence of four sets of autosomes in P_1.

Two other F_1 intersexes of a somewhat different type (table 6, column 8) were in all probability 4X 3A flies. The wings showed disturbances which are commonly found with an unbalance between X chromosomes and autosomes (as in 3X 2A and 2X 3A flies); the eyes were very slightly bar, not so distinctly bar as in $3n$ flies. Both were sterile. Lastly, among the F_1 females was one fly which, though much smaller, resembled the $4n$ mother in that the eyes were faintly bar or nicked, while the wings were normal. She produced one fly which was heterozygous bar of the degree typical for the $3n$ flies, and she herself was probably $4n$. BRIDGES has

TABLE 7

P_{11} heterozygous hairy-wing heterozygous bar $\left(\frac{yHw}{B}\ \frac{m}{m}\right)$ *4n ♀: Test for two attached and two free X chromosomes by 100 percent of partial non-disjunction in F_3.*

$F_1(3n)$	$F_2(2n)$	F_3		
$\frac{\pm H_w\ B^*}{+\ +}$	Attached X's	♀♀ Crossovers		♂♂
20b	35d, $\frac{B}{+}$	42 $\frac{B}{+}$	3*m* $\frac{B}{+}$ 1*m*	Like father
20c	40b, $\frac{H_w\ B}{+\ +}$	$\frac{\pm H_w\ B}{+\ +}$	1 $yH_w\frac{B}{+}$	" "
	40e, *fB*	18 *fB*		" "
	36g, $\frac{B}{+}$	12 $\frac{B}{+}$	1 wild-type	" "
$F_2(3n)$	58e, $\frac{H_w\ B}{+\ +}$	$\frac{\pm H_w\ B}{+\ +}$	1 $yH_w\frac{B}{+}$	" "
	58f, $\frac{H_w\ B}{+\ +}$	$\frac{\pm H_w\ B}{+\ +}$	?	
20h, $\frac{H_w\ B}{+\ +}$	58g, $\frac{H_w\ B}{+\ +}$	0		15 " "
38y	58h, $y\frac{H_w\ B}{+\ +}$	0		14 " "
20s	46a, $\frac{H_w\ B}{+\ +}$	21 $\frac{\pm H_w\ B}{+\ +}$	2 $\frac{H_w}{+}mfB_1 2\frac{H_w}{+}m$	" "
	46j, $\frac{H_w\ B}{+\ +}$	$\frac{H_w\ B}{+\ +}$	2 $yH_w\frac{B}{+}$ 1 $\frac{H_w}{+}$	" "
	46l, $\frac{H_w\ B}{+\ +}$	27 $\frac{\pm H_w\ B}{+\ +}$	2 $yH_w\frac{B}{+}$ 1 $\frac{H_w}{+}$	" "
20t	48a, $m\frac{B}{+}$	1 $m\frac{B}{+}$		" "
	48d, $\frac{B}{+}$	2 $\frac{B}{+}$		" "
$\frac{H_w}{+}$ 20q	44x, yH_w	yH_w	1 $yH_w\,m$	" "
	44y, yH_w	yH_w		" "

Wild-type	Separate X's:	Mate	Regular	Non-disjunctional	Regular	Non-disjunctional
20e	37a, *m* ×	*vfB*	69 $\frac{B}{+}$	1*m*	65*m*	2*vfB*
20f	37j, *m* ×	*v*	Wild-type	1*m*	*m*	*v*?
	37k, *m* ×	*vfB*	40 $\frac{B}{+}$	1*m*	46*m*	4*vfB*
	37l, *m* ×	wild-type	Wild-type	1*m*	*m*	1 wild-type
20σ	40b, *m* ×	wild-type	Wild-type	1*m*	*m*	

* Phenotype.

previous records (unpublished) of two $4n$ females arising from $3n$ mothers with separate X chromosomes.

NON-DISJUNCTION IN MALES

If non-disjunction of one or more chromosomes should occur in a male at such a time that the sperm would have an increased number of chromosomes, the affected sperm would presumably produce an embryo if it fertilized an egg whose chromosomes were of a number to make a viable combination with those of the sperm. For example, if a sperm had two X chromosomes and fertilized a common egg with one X chromosome the result would be a poorly viable 3X 2A super-female. But if the male had been mated to a female with attached X's, the chances are even that the 2X sperm would fertilize an egg with a Y chromosome, and a viable combination would result, producing a patroclinous female with a Y chromosome. Patroclinous females should then be more numerous in 100 percent non-disjunctional lines than in the common lines with separate X's. As a matter of fact they have been more often reported from non-disjunctional lines although very little of the work on Drosophila has been done with those lines.

Another viable combination would be a sperm with diploid first, second and third chromosomes and an egg with a Y chromosome, resulting in a patroclinous intersex, such, perhaps, as the two or three apricot intersexes already referred to as offspring of an attached-X female. The same kind of sperm with an ordinary-X egg would produce a $3n$ female. The same kind of sperm fertilizing an egg with attached X's and two sets of autosomes from a $3n$ mother would produce a $4n$ female; the genetic records of the $4n$ female just described show that it might have been produced in this way. Sperms with diploid autosomes and a single X chromosome would produce, if they fertilized eggs with attached X's, the kind of $3n$ females found in non-disjunctional lines; those recorded were not marked to show whether the doubled autosomes came in with the egg or with the sperm. Sperm with diploid second or third autosomes, but not both, could not make a viable combination with any regular egg.

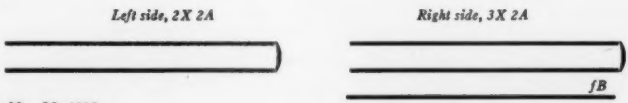
The mosaic from which the double-yellow stock originated was probably at the outset a super-female which became in part a fertile female by the elimination of an X chromosome. The fact that the sons of the mosaic were sterile is in accord with the explanation, since the absence of a Y produces sterility. MORGAN and BRIDGES (1919) have shown that a female fly may become in part male by elimination at some stage of development of one X chromosome, and similarly the loss of an X from one region of a 3X 2A embryo would produce a fly with the characters

and behavior of the mosaic. The female region was patroclinous, for the father was yellow and there was no yellow in the parentage of the mother. The attachment of the chromosomes can be formally explained if at some division of the chromosomes the daughter halves failed to separate completely.

It may be recorded here that two other mosaics, with a yellow female region, and two yellow females were found in related cultures of the same pedigree as that of the first mosaic. The mosaics showed characters carried by the X chromosomes of the mother, but in these flies the results could not be explained by a single elimination. If in the yellow females there was a region with a maternal X chromosome, it failed to show, although the diagnostic character was a general body color. One of the yellow females and one of the mosaics bred as yellow females with separate X chromosomes.

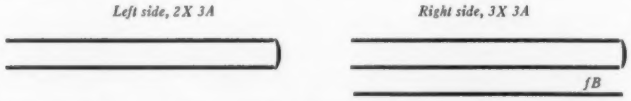
COMPOSITES

Three different 3*n* females each produced a fly which was mosaic for a sex-linked character. In one instance (65a) the mother had three X chromosomes without mutant differentials and had the differentials for dominant curly wing and recessive black body color in one of her three second chromosomes. She was mated to a forked bar homozygous black male. The mosaic offspring was altogether not black, its left eye was not bar, but somewhat rough and bulging. The right eye was rather broad heterozygous bar. The left wing was not-curly and normal. The right wing was too small and irregular to admit of judging the character curly, and the last right leg was abnormal. There were no sex combs and therefore the region including the fore legs, at any rate, showed neither intersex nor male constitution; the abdomen also was entirely female. The mosaic can be explained by elimination. If an egg with the attached chromosomes and one set of autosomes had been fertilized by X sperm which carried bar, a heterozygous bar super-female would have been produced, and such a 3X 2A condition might account for the abnormalities of the right side, and for the somewhat broad type of bar eye. After elimination of the free chromosome carrying bar, a region like that of the left side would develop, where the eye was wild-type and the wing was normal. The left side showed the regular 2*n* characteristics (except that the eye was slightly irregular) and the mosaic was probably a composite of super-female and female.



The alternative explanation of a binuclear origin also fits with the facts: if two nuclei had each a pair of attached chromosomes, one fertilized by X sperm would give conditions found in the right side, the other with Y sperm would give those found in the left side. Only the region including the left side could be fertile and it was hoped that the gonads might be included in it and that the presence or absence of a Y chromosome in that region could be demonstrated by testing the fertility of male offspring. By this test one or the other of the alternative explanations could be disproved. Unfortunately, the fly failed to breed. Evidence for the binuclear explanation from the second-chromosome characters was negative.

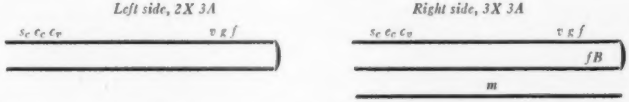
In another culture of the same *3n* stock a fly mosaic for bar was found (72a), which can be explained if it was a composite of intersex and *3n* female. The mother was heterozygous bar because her free X chromosome carried bar; this did not therefore affect her *3n* offspring which received from their mother an attached pair of chromosomes with no mutant differentials. The mother had one second chromosome which carried curly black, one with black and one wild-type, and was mated to forked bar black. One of her offspring was black all over with both wings curly; there were no sex combs. The left eye was wild-type and the right eye was broad heterozygous bar, which, with the normal condition of the fly, suggested that it was in the main a *3n* female. Elimination of the paternal X carrying forked bar would leave an intersex (2X 3A) region and might account for the round eye, which was rough as in an intersex. If so, the intersex region included only the left part of the head, because all of the right side and the left side of the thorax with no sex comb and the left wing were normal, and the abdomen was normal female. The fly was mated and proved to be *3n* in the region which included the genital organs.



As before, the binuclear explanation would apply and the evidence from the autosomes is negative.

A *3n* fly (No. 10a), in which the left side of the thorax was intersex, was the offspring of a heterozygous bar *3n* female. Mated to a wild-type male the composite bred as a *3n* female in which one of the attached chromosomes carried the differentials for the combination z-ple, and the other those for forked and bar. This had been the constitution of her mother's attached chromosomes, which she inherited without crossing

over. Her father was miniature. The eyes were broad heterozygous bar; the right side of the thorax with no sex comb, the right wing and the female abdomen showed no recessive characters and were normal, as expected in a $3n$ fly of the given parentage. The genetic behavior was also in accord. The left side of the thorax, on the other hand, was forked, and the left wing had thickened veins and was shorter than the right and somewhat compressed as in an intersex; also, there was a sex comb. If the paternal X chromosome had been eliminated, the region affected should be intersex and should be forked because the two attached chromosomes were homozygous for forked. The mosaic of intersex and female characteristics and of phenotypic characters and the genetic behavior are all in accord with the interpretation that a $3n$ female became composite in regard to the number of chromosomes by elimination of one X chromosome.



Again there is no evidence which discriminates between this and the binuclear interpretation.

OTHER EXCEPTIONAL FLIES OF THE DOUBLE-YELLOW STOCK

The $3n$ female, No. 182, was related to the non-disjunctional culture already referred to as No. 184 (table 1), in which there were many offspring, both male and female, with a detached yellow-bearing chromosome. At about the same time there were found three other heterozygous bar females in different related cultures of the same stock of double-yellow females and bar males. Two of them gave the regular results for females with a (detached) yellow-bearing chromosome and a bar chromosome. The third, No. 185, gave mixed results. She was backcrossed to a bar male and the offspring were 26 bar, 39 heterozygous bar, 3 yellow and 3 wild-type females, and 42 bar, 33 wild-type, 5 yellow and 13 yellow bar males.

The three yellow F_1 females were tested and proved to have attached X chromosomes, both carrying yellow. The three wild-type F_1 females were tested and proved also to have attached X chromosomes: in one of them one chromosome was wild-type and one carried yellow; the wild-type chromosome would have resulted if crossing over had taken place with a bar chromosome, the only kind present except yellow. In the other two wild-type F_1 females, neither of the attached chromosomes carried yellow; if crossing over was the means by which yellow was lost, both strands of a

free chromosome had crossed over to attached strands, an occurrence not found in the records of No. 182. There is no evidence that two free chromosomes were present.

Three of the heterozygous bar F_1 females were tested and all had two separate X chromosomes. One of the females had a yellow-bearing and a bar chromosome as found in females with a detached X chromosome from the mother and an X chromosome from the father. But the other two heterozygous bar F_1 females both had a wild-type chromosome and one carrying bar. If their mother had herself been derived from a detached-X egg, these two daughters would be explained as receiving a crossover (wild-type) chromosome from their mother, but since she had six daughters with attached X's (the three yellow and three wild-type described above), she herself must have had attached X's.

The wild-type chromosomes then remain to be explained; they appeared not only in two out of three tested heterozygous bar daughters, but in 33 sons. It has been shown that the mother had attached X's and that yellow had been lost in some daughters with attached X's, possibly by crossing over with a free chromosome carrying bar. A wild-type free chromosome might have resulted from a double crossing over with an attached X chromosome by which the section with the locus for bar in the free chromosome was replaced by a section carrying the wild-type allelomorph from an attached X. But the offspring with a wild-type chromosome are far too numerous to be the result of so rare a kind of crossing over.

The loss of yellow from some of the attached chromosomes suggests that crossing over had taken place in them, which is known only in *3n* flies. Whether or not the mother was *3n* the data do not show; if she was, it may well be that intersexes among her offspring were overlooked; but even allowing for intersexes in the classes where they might have occurred, the count of the F_1 offspring is far from typical, as well as the kind of crossing over that appears to have taken place. No explanation adequate for other exceptional flies will account for the various results found in F_1 of No. 185.

Five other exceptional females of double-yellow stock that have given results different from any others so far known will not be described here.

A new occurrence of a *3n* female (No. 199) which was the wild-type offspring of a double-yellow female and an apricot, cut, vermilion, forked male, has recently been found. The *3n* condition was recognized by the characters and kinds of F_1 offspring, including intersexes. Further evidence was given by the results in F_2 of breeding a wild-type daughter

to a forked bar male. Not being bar, the wild-type daughter must have received her two X chromosomes from her mother,—the attached chromosomes,—and not being yellow, there must have been in the mother a third X chromosome with which one yellow attached chromosome had crossed over, namely, the apricot-bearing chromosome. The daughters of the wild-type female were of the expected classes; the largest class was wild-type, the smaller classes were yellow and apricot.

SUMMARY

In lines of *Drosophila melanogaster* with two attached X chromosomes, the offspring are daughters like their mother and sons like their father, in a 1:1 ratio. The females have, in addition to the two attached X chromosomes, a Y chromosome. One-fourth of the offspring are expected to be super-females (XX X) and, though poorly viable, a few in fact appear.

Occasionally the attached chromosomes break apart and an egg receives a single X chromosome and gives the same results as an ordinary egg. There is no evidence that the breaking apart of attached chromosomes depends on an inherited factor.

Rarely individuals appear that are patroclinous females, having two paternal X chromosomes and a Y chromosome. They develop presumably from a Y egg fertilized by non-disjunctional XX sperm.

A very few single intersexes have been found among the offspring of females with attached X chromosomes.

Triploid females also rarely occur in the cultures. They have two attached X chromosomes and a free X chromosome. Crossing over takes place between the attached X's and the free X. The resulting chromosomes are, on the one hand, a free chromosome with a piece of the left end of one chromosome of the attached pair, and, on the other hand, attached chromosomes with an equal piece of the left end of the free chromosome crossed over to one of them.

From crossing over between marked chromosomes it is proved that the attached chromosomes are joined by their right ends.

From non-disjunctional lines in which the attached chromosomes are not alike, it is found that crossing over may take place between the attached X chromosomes.

A tetraploid female with two attached X chromosomes and two free X chromosomes was found among the offspring of a triploid female.

Three composite flies have been found among the offspring of triploid females. They were composed of a super-female and a female region, or of a triploid and an intersex region.

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MOSAIC PERICARP IN MAIZE[1]

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INTRODUCTION

Mosaic pericarp is a coarse type of color variegation in the pericarp of maize. It is typical of variegations in that it is inconstant, exhibiting a high degree of variability in pattern, and giving rise to an inconstant dilute self-colored type (orange) and to stable colorless and self-red strains. In the present paper are described color and pattern variations that originated in pedigreed cultures and from a common source, but particular attention is given to the frequency of the occurrence of these variations and to the gene mechanism responsible for the change from one color or pattern to another.

PREVIOUS STUDY OF MOSAIC PERICARP

HAYES (1917) studied the mosaic pericarp patterns by making continuous selections of the various types for a number of years, and then crossing them with one another to determine their genetic relationships. The following genetic types were isolated: (1) self-red; (2) mosaic patterns ranging from ears in which nearly all of the seeds were heavily striated to ears in which only a few seeds showed red stripes; (3) dilute self-color

[1] This investigation was made in the Department of Field Crops, MISSOURI AGRICULTURAL EXPERIMENT STATION.

or orange (called very slight pattern by HAYES); and (4) colorless. It was found that the heavily striated ears tended to produce a progeny with more ears with a heavy pattern than was obtained in the progeny of the few-stripe ears, but the range of variation was not affected by continuous selection. The F_1 plants from the cross self-red × mosaic are reported as having had mosaic pericarp but more intense than the mosaic parent, and when such F_1 plants were self-pollinated F_2 progenies in the ratio of 1 typical mosaic:2 intense mosaic:1 self-red were produced. The cross self-red × pattern was found to give self-red ears in the F_1, and a ratio of 3 self-red : 1 pattern in the F_2 generation. The cross pattern × mosaic gave F_1 progenies with a greater range of variation than either parent and also a considerable percentage of bud-sport ears. From these results HAYES concluded that certain combinations produce germinal instability and that the factors for self-red, mosaic, very slight pattern (orange), and colorless, form a series of multiple allelomorphs.

MATERIAL AND METHOD

The strains of maize with mosaic pericarp that were used in the experiments reported were furnished by Doctor E. G. ANDERSON who obtained them from Doctor H. K. HAYES. All of the self-red strains that I had in my pedigreed cultures, as well as the self-red strain which was reported by HAYES as being recessive to mosaic, were used in the study of the relation of self-red to mosaic.

The material used for study was produced in a manner similar to that used in a study of the common calico type of variegation which was reported in a previous paper (EYSTER 1924). The variability in both color intensity and color pattern indicates that the gene concerned is inconstant. In order to study a particular variable gene it is necessary to have it associated with another gene which is stable and also allows the changes in the variable gene to come into expression. Accordingly, strains with mosaic pericarp and cob color were crossed with strains having colorless pericarp and red cob. This cross may be expressed factorially as follows: $MM \times WR$.[2]

The F_1 plants from the cross indicated above have mosaic pericarp and red cob and have the genetic constitution $\frac{MM}{WR}$. The F_1 plants, when backcrossed with strains having colorless pericarp and white cob, of the

[2] A single gene determines the development of pigment in both pericarp and glumes of the cob. For example, the gene *WR* produces colorless pericarp but red cob, while the gene *WW* causes both pericarp and cob glumes to be without pigment.

constitution $W\ W$, give progenies of two sorts as regards pericarp color, in approximately equal numbers: (1) plants with ears having colored cob glumes and pericarp, due to the gene MM in one of its allelomorphic forms as follows: mosaic cob and pericarp of the constitution $\frac{MM}{WW}$, self-red cob and pericarp of the constitution $\frac{RR}{WW}$, orange cob and pericarp of the constitution $\frac{OO}{WW}$; and (2) plants with ears having colorless pericarp and red cob, of the constitution $\frac{WR}{WW}$. The first group of ears has the gene for mosaic pericarp associated with a highly stable gene for colorless pericarp, so that any changes undergone by the variable gene for mosaic pericarp are expressed as color or pattern variations. The ears of the second group have the stable gene for colorless pericarp. Since the gene for mosaic pericarp is not involved, the ears were discarded. The red cob identifies the WR gene and makes it possible to eliminate it from the study.

The ears of the first group were separated into classes according to color intensity and color pattern of the pericarp. The ears of each class were then examined individually for color and pattern changes that extended over patches larger than a single kernel. Finally the kernels were shelled off the cob and the individual kernels separated into arbitrary classes according to their color intensity and color pattern.

DESCRIPTION OF THE MOSAIC PATTERNS

The inconstancy of the mosaic patterns was described by HAYES (1917), who found that the mosaic strains give in their progeny a range of variability from ears with only a few seeds with deep-red stripes to ears in which nearly all of the seeds are quite heavily covered with red striations. Pattern changes in the progenies of self-fertilized mosaic strains may be due to the segregation of genes as well as to changes in the genes themselves. By crossing the mosaic strains with the colorless strains, as already described, it was possible to eliminate variations due to the segregation of genes for mosaic pericarp of different patterns. The pattern variations in mosaic strains that are heterozygous for colorless pericarp must be considered either different expressions of the same gene under different conditions, or distinct patterns due to some physical or chemical change in the gene itself.

The ears shown in figures 1 to 3 illustrate the more common pericarp variations that originated in pedigreed cultures from a single mosaic ear.

Ear a, figure 1, has a very light mosaic pattern, having only a few streaks of color on the entire ear. The ears b, c and d of figure 1 are typical of the heavier patterns, ranging from ears with fine streaks of red, more or less uniformly distributed, to ears in which nearly all of the seeds are heavily covered with bands of red. Ear e, figure 1, is from a self-red strain which originated from a heavy-mosaic type.

Frequently two or more patterns may appear on the same ear. Ear a, figure 2, has the light mosaic pattern over all of its surface except a large patch near the base, where the pattern is coarse mosaic. The ear b, figure 2, appears to have three distinct patterns: a fine rather uniform pattern

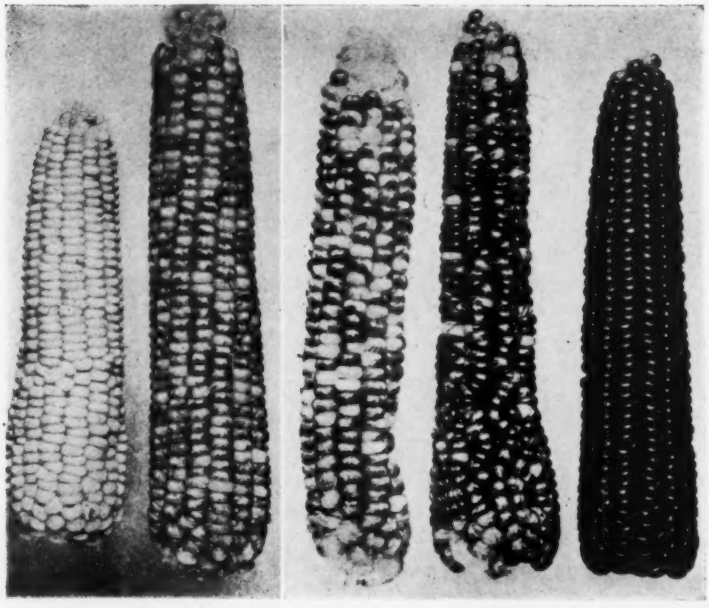
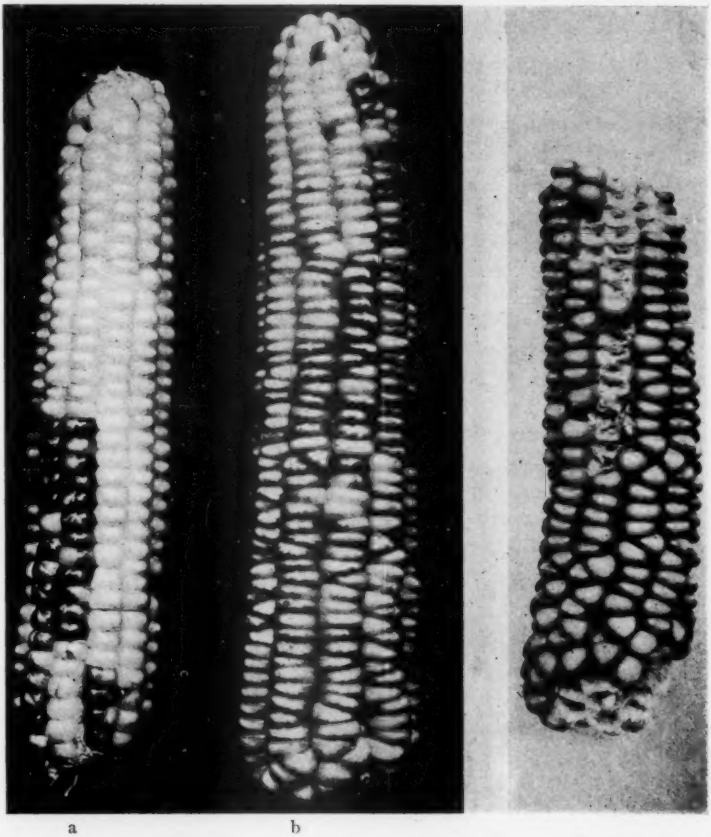


FIGURE 1.—Variations in mosaic pattern: a represents light mosaic; b, c and d illustrate heritable differences in heavy mosaic; e is a self-red ear of a strain that originated from mosaic.

(like that of ear b, figure 1) at the base, a heavy coarse pattern (like that of ear d, figure 1) on one side of the tip, and a light pattern (like that of ear a, figure 1) on the other side of the tip.

The ear illustrated in figure 3 has a dilute-red or orange-colored pericarp (called "very slight pattern" by HAYES). It is significant that a dilute-self-color or orange type should appear in the mosaic strains and resemble

in all respects the orange type found in the variegated series described elsewhere (EYSTER 1924) and the lilac color of petals in *Hesperis matronalis*, which, according to DE VRIES (1910), occurs in the variegated strains.



a b

FIG. 2

FIG. 3

FIGURE 2.—Ears with more than one genetic type of mosaic: a has light mosaic over most of its surface, but a large patch of heavy mosaic at the base; b has a fine mosaic pattern at the base, a light pattern on one side of the tip, and a heavy pattern on the other side of the tip.

FIGURE 3.—An ear with the dilute-red or orange type of pericarp.

INHERITANCE OF THE MOSAIC PATTERNS

By intercrossing the various color and pattern types which belong to the mosaic series, HAYES (1917) found them to be multiple allelomorphs.

Since all of the types have originated in the course of a few generations, and sometimes within a single generation, from a single parent plant, it is to be concluded, at least as a working hypothesis, that they represent diverse changes in a fundamental pericarp color gene. In connection with the preparation of suitable material for the study of the frequency and direction of color and pattern changes, intercrosses were made between most of the types, which showed the following genetic relationships:

Dominant	*Recessive*
Self-red	All other colors and patterns
Heavy mosaic	Light mosaic, orange, colorless
Light mosaic	Colorless
Orange	Colorless

So far as tests have been made, the results of the intercrosses agree with those reported by HAYES, with the important exception that self-red is not recessive to mosaic. Crosses were made between mosaic and a large number of self-red strains, including the self-red strain obtained from Doctor HAYES and reported by him as being recessive to mosaic, and in no case were the F_1 ears other than deep self-red. A large number of these F_1 plants were grown and many of them were backcrossed with a strain having colorless pericarp and white cob. The progenies from these backcrosses consisted of mosaic ears and self-red ears in approximately equal numbers, thus showing that the self-red F_1 ears were heterozygous for the mosaic pattern. The relation between orange and mosaic was not tested, but, according to HAYES, the orange type of pericarp is recessive to all the mosaic patterns.

Inasmuch as many of the ears that were classed as self-red by HAYES are recorded (HAYES 1917, table 1) as having produced some mosaic ears in their progenies when self-pollinated, it is possible that they were actually heavy mosaics. A cross between such a phenotypically red but genotypically deep-mosaic strain and a medium-mosaic strain would be expected to give the results observed by HAYES, namely, deeply striated F_1 ears intermediate in pattern as compared with the parent strains, and an F_2 progeny of 1 heavy mosaic (resembling self-red in appearance): 2 intermediate mosaic: 1 typical mosaic.

FREQUENCY OF COLOR AND PATTERN CHANGES

Mention has already been made of the common origin of the variations in color and pattern that have been described. These variations reproduce themselves in large measure, but change more or less frequently from one pattern or color to another.

Light mosaic

The very light mosaic (figure 1, ear a) is a very distinct pattern and is easily distinguishable from the more common heavy patterns.

A light-mosaic plant which was heterozygous for colorless pericarp and red cob was backcrossed with a plant having colorless pericarp and white cob, and produced a progeny, grown under pedigree number 3808, of 100 ears with colorless pericarp and red cob and 92 ears with mosaic pericarp. This is a close approximation to the 1:1 ratio expected. Of the mosaic ears 85 had the light pattern of the parent plant; one ear had three-fourths of its area changed to heavy mosaic; one ear was heavy mosaic over about one-eighth of its surface with the remainder of its surface medium mosaic; three ears had a medium-mosaic pattern; one ear had a patch of 34 kernels changed to medium mosaic, and one ear had a patch of 44 kernels changed to medium mosaic.

A similar progeny, grown under pedigree number 3807, yielded 120 mosaic ears. Of these 115 had the light pattern of the parent plant; three ears had more than half of their surface changed to medium mosaic; one ear had a patch of 12 kernels changed to medium mosaic; and one ear had a patch of six kernels changed to orange pericarp.

A summary of the changes from light mosaic to the heavy patterns as observed in four progenies is given in table 1.

TABLE 1

PEDIGREE	KERNELS LIGHT MOSAIC	CHANGES TO HEAVY PATTERNS, IN KERNELS					
		Band	¼	½	¾	1	>1
3807	29,237	0	0	0	0	0	4
3808	24,481	0	1	0	1	0	7
3811	5,438	1	0	0	0	2	0
4037	8,885	1	0	0	0	0	0
Total	68,041	2	1	0	1	2	11

The seventeen changes from light mosaic to heavy mosaic indicated in the above summary ranged in size from a small segment of a single kernel to patches covering an entire ear. As there were 68,041 with no change in pattern, the frequency of change from light to heavy mosaic may be said to be about one in 4000 kernels. The fact that most of the observed changes affected large areas would seem to indicate that they occur more frequently in early development than in the later stages.

Among the 68,041 light-mosaic kernels were also found 132 changes that appeared to be self-red, but only one of the changes was as large as one-half of a kernel, as may be seen in the following summary:

Unchanged (light mosaic)	68,041 kernels
Color changes to red	
Band (small segment)	127 kernels
One-fourth surface of a kernel	4 kernels
One-half surface of a kernel	1 kernel

This would be a frequency of change from light mosaic to red of one in a little more than 500 kernels. It must be admitted that while these color changes appear to be self-red they may in reality be heavy mosaic. So far as tests were made none of these changes was inherited, evidently due to the fact that only somatic tissue was affected.

Also among the 68,041 light-mosaic kernels were found 42 changes to dilute-red or orange pericarp, ranging in size from a narrow segment of a kernel to the entire surface of a kernel, as shown in the following summary:

Unchanged (light mosaic)	68,041
Changed to orange pericarp	
Band (small segment)	36
One-fourth surface of a kernel	3
One-half surface of a kernel	1
Three-fourths surface of a kernel	1
Entire kernel	1

According to these observations the frequency of change from light mosaic to orange is one in about 1620 kernels.

Heavy mosaic

The most striking characteristic of the more common mosaic types is the coarse, heavy pattern with a relatively large number of colorless kernels scattered over the ear. There is great variability in pattern depending upon the size and distribution of the colored segments. Some of the more common patterns are illustrated in figure 1, ears b, c and d.

TABLE 2

PEDIGREE	EARS WITH MOSAIC PATTERN			
	Heavy	Medium	Light medium	Light
1536	5	26	28	0
3799	19	27	3	2
4035	5	67	15	5
4109	14	75	10	5

The heavy mosaic patterns are extremely inconstant, as was shown by HAYES (1917). A progeny includes, in addition to the parental type, both

TABLE 3

PEDIGREE	TYPE	FREQUENCY OF CHANGE FROM HEAVY MOSAIC TO																
		Medium mosaic Areas expressed in kernels									Light mosaic Areas expressed in kernels							
		¼	½	¾	1	2	3	4	5	>5	¼	½	¾	1	2	3	4	5	>5
4031	1640	318	185	106	56	16	6	5	3	2	205	99	33	20	0	0	0	0	0
4035	3365	480	243	86	60	10	5	2	1	0	226	126	50	21	5	2	1	0	0
4109	1210	638	251	149	64	26	11	0	3	1	216	119	51	21	5	2	1	1	2
Total	6215	1436	679	341	180	52	22	7	7	3	647	344	134	62	10	4	2	1	2
Number per 1000	612.	141.5	67	33.5	18	5.1	2.2	.7	.7	.3	64	34	13	6.1	1.0	.4	.2	.1	.2

PEDIGREE	TYPE	FREQUENCY OF CHANGE FROM MEDIUM MOSAIC TO																
		Fine medium mosaic Areas expressed in kernels									Light mosaic Areas expressed in kernels							
		¼	½	¾	1	2	3	4	5	>5	¼	½	¾	1	2	3	4	5	>5
4035	4015	1077	561	187	230	16	5	1	0	0	536	412	147	112	6	1	2	0	5
4109	5257	2886	1364	653	484	102	40	28	16	44	1427	680	283	208	30	17	5	3	8
Total	9272	3963	1925	840	714	118	45	29	16	44	1963	1092	430	320	36	18	7	3	13
Number per 1000	444.	190.	92.	40.	34.	5.7	2.2	1.4	.8	2.1	94.	52.4	20.6	15.4	1.7	.9	.3	.2	.7

lighter and darker patterns, so as to produce a frequency distribution with the parental type as the modal class. Table 2 gives a summary of a number of progenies which had the mosaic gene associated with a gene for colorless pericarp.

An effort was made to determine with some degree of accuracy the frequency of changes in the heavy patterns. In table 3 are given the frequencies of changes from heavy mosaic to medium mosaic, heavy mosaic to light mosaic, medium mosaic to fine medium mosaic, and medium mosaic to light mosaic. The coarseness of pattern makes even an arbitrary classification extremely difficult, but high frequencies of change are apparent. The frequencies (taken from table 3) of such changes as affected a whole kernel or larger areas are given in table 4.

TABLE 4

PATTERN CHANGE	NUMBER OF CHANGES PER THOUSAND KERNELS EXAMINED
Heavy to medium.....	27
Heavy to light.....	8
Medium to fine medium	46
Medium to light.....	19

These results indicate that the medium mosaic patterns change to lighter patterns about 1.9 times as frequently as does the heavy pattern studied. The extremely heavy pattern which approaches self-red in appearance changes to lighter patterns or colorless much less frequently than the heavy patterns included in this study. Just as the light-mosaic strains include a relatively large number of ears that appear colorless, the extremely heavy-mosaic types produce many ears that appear self-red. Apparently the frequency of change of self-red increases from light to the heavy patterns, while the changes to lighter patterns and colorless decrease as the patterns become more and more heavy.

The medium- and heavy-mosaic types were observed to change to dilute red or orange with the frequencies shown in table 5.

TABLE 5

PEDIGREE	MOSAIC KERNELS EXAMINED	ORANGE KERNELS OBSERVED
4031	2,702	0.5
4035	11,996	0
4109	13,242	1
Total	27,940	1.5

The frequency of change from heavy mosaic to orange, based on the above observations, is one in about 18,600 mosaic kernels.

Orange

Orange pericarp is a dilute self-color that has been observed to originate from the mosaic strains. The frequency of the change from mosaic to orange is summarized in table 6.

TABLE 6

MOSAIC PATTERN	NUMBER OF KERNELS EXAMINED	CHANGES TO ORANGE	CHANGES TO ORANGE PER THOUSAND KERNELS
Heavy.....	27,940	1.05	0.054
Light.....	68,041	42	0.620

The color intensity of the orange kernels varies from whitish to red. The progeny ears of a number of orange strains heterozygous for colorless pericarp were separated into arbitrary color-intensity classes ranging from whitish (class A) to red (class F) with the results shown in table 7.

TABLE 7

PEDIGREE	INTENSITY OF ORANGE COLOR (WHITISH) A	B	C	D	E	(RED) F	EARS MOSAIC
4026	3	9	14	24	13	5	1
4037	5	11	32	17	13	7	0
4124	11	19	31	14	4	2	0
4126	0	2	7	23	16	4	0

This fluctuating variability in color intensity is similar to that in the orange pericarp that originates from the calico type of variegation and to the lilac petal color in *Hesperis matronalis* to which reference has been made before.

Orange pericarp has been observed to change to mosaic and to what seems to be self-red, with the frequencies recorded in table 8, a summary of which is given in table 9. The changes from orange to mosaic and red are distinct and clear-cut, so that it was possible to get a good measure of the frequency of these changes. The frequency of change from orange to mosaic increases with color intensity up to class D, which has the highest frequency, 92.75 changes per thousand orange kernels examined. In the darker classes, E and F, the frequency of change from orange to mosaic

TABLE 8

PEDIGREE	TYPE	COLOR CHANGES TO									
		Mosaic								Red	
		Line	Band	¼	¾	½	¾	1	2	Line	Band	¼
Light A											
4026	532	18	7	..	..	1	(1 entire ear)			..	..	..
4037	1,358	60	..	..	1	..				..	..	..
4124	1,633	76	..	..	..	..				..	..	..
Total	3,523	154	7	..	1	1	..	..	..	..	..	..
B											
4037	2,709	66	10	..	..	..	..	..	..	..	..	..
4026	1,772	400	31	2	1	1	..	..	..	1	..	..
4124	3,543	218	6	..	..	..	..	..	..	..	..	..
4126	597	6	..	..	..	..	..	..	..	..	..	..
Total	8,621	690	47	2	1	1	..	..	..	1	..	..
C											
4026	2,789	458	46	3	1	2	..	1	..	8	2	..
4037	9,600	380	27	..	..	..	..	1	..	3	..	..
4124	6,683	262	8	..	..	..	..	..	..	1	..	..
4126	2,040	63	9	..	..	..	..	..	..	1	2	..
Total	21,112	1163	90	3	1	2	..	2	..	13	4	..
D											
4026	4,884	830	48	11	4	5	..	1	..	9	6	2
4037	4,732	535	5	2	2	..	..	1	..	4	1	..
4124	3,179	34	4	..	.	..	..	..	..	1	..	..
4126	5,409	311	51	4	6	4	2	4	..	3	2	1
Totals	18,204	1710	108	17	12	9	2	6	..	17	9	3
E											
4026	3,221	430	33	9	4	1	2	2	..	33	4	1
4037	3,972	71	7	2	1	..	1	1	..	5	1	..
4124	995	7	..	..	..	..	..	..	..	..	..	..
4126	3,963	175	38	2	2	1	1	1	..	1	1	..
Totals	12,151	683	78	13	7	2	4	4	..	39	6	1
Dark F											
4026	914	92	3	..	1	1	1	..	2	10	4	..
4037	1,965	29	6	..	..	1	..	..	..	11	4	..
4124	810	4	..	..	..	..	..	..	..	..	..	..
4126	1,190	13	7	..	..	..	..	..	..	4	1	..
Totals	4,879	138	16	..	1	2	1	..	2	25	9	0

decreases as the color intensity increases. Evidently there is a correlation between color intensity and gene stability.

TABLE 9

INTENSITY OF ORANGE	KERNELS UNCHANGED (ORANGE)	NUMBER OF CHANGES TO		NUMBER OF CHANGES PER THOUSAND KERNELS EXAMINED	
		Mosaic	Red	Mosaic	Red
A (whitish)	3,523	163	0	44.22	0.00
B	8,621	741	1	79.14	0.11
C	21,112	1216	17	54.42	0.76
D	18,204	1864	29	92.75	1.44
E	12,151	791	46	60.90	3.54
F (red)	4,870	160	34	31.62	6.71

The frequency of change from orange to red varies directly with color intensity of the orange, from 0.11 in class B to 6.71 in class F per thousand orange kernels.

Colorless

A colorless strain was found that originated in a pedigreed culture of a mosaic strain. Relatively large progenies of this strain have been grown for three generations and over a thousand ears have been examined, but no traces of color have been observed. It is to be concluded that this colorless strain has lost the instability of the mosaic strain from which it originated.

Self-red

A large number of what seemed to be self-red ears that originated from the mosaic strains have been tested, but practically all of them proved to be genotypically heavy mosaic.

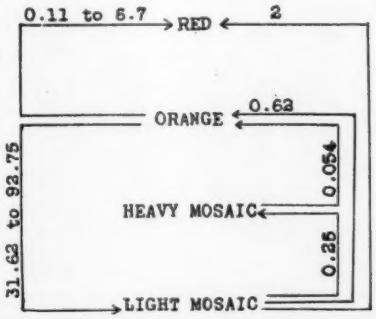


FIGURE 4.—A diagram to show the relative frequencies of pericarp color and pattern changes expressed in number of changes per thousand kernels.

At least one strain has been found (figure 1, e) that originated from mosaic, which, so far as tested, breeds true for the self-red color.

Summary of the color and pattern changes

The relative frequencies of the various color and pattern changes that have been described above are shown in the diagram in figure 4.

THE NATURE OF THE CHANGE FROM ORANGE TO MOSAIC

The character intermediate between the contrasting characters of the variegation, which is dilute red or orange in maize, is extremely interesting because of the light it throws on gene structure and the nature of variegation. In orange strains heterozygous for colorless pericarp the orange color

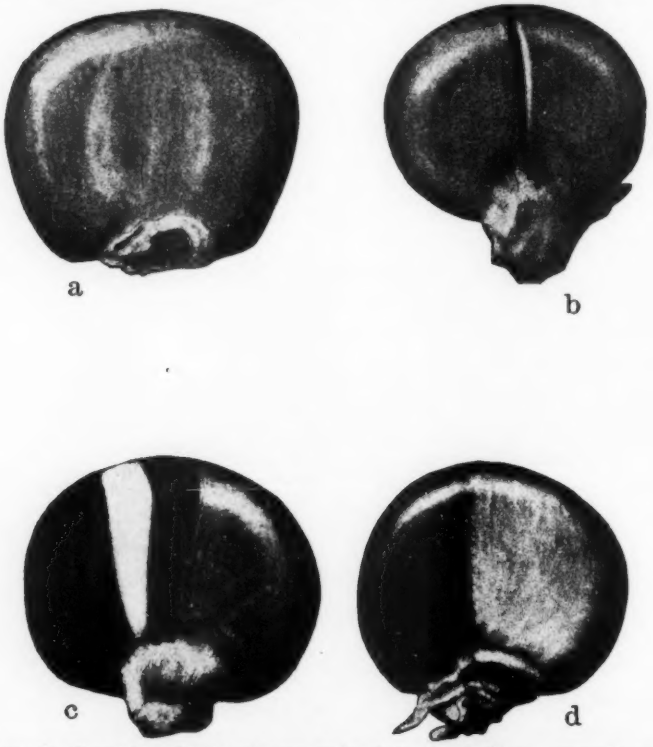


FIGURE 5.—Orange kernels with color segregations into red and white: a represents a typical orange kernel; b, c and d have the orange color replaced by equal and adjacent segments of red and white. Drawings by GEORGE KLINE.

breaks up into its component colors, red and white (colorless). In figure 5 are shown orange kernels with color segregation. Kernel a is a typical orange kernel with no color change. In kernel b, a small segment of the surface has the orange color replaced by a segment of red adjacent to an equally large segment of colorless pericarp. It is quite evident that the gene for orange pericarp was divided by a mitosis late in development in such a way that the pigment-producing and non-pigment-producing parts of the gene were separated in daughter cells. The red and white segments represent the cell progeny of the daughter cells with and without pigment-producing gene substances, respectively. The kernels c and d, figure 5, show similar color segregation, except that larger parts of the kernel are involved, evidently due to the breaking up of the orange gene at an earlier stage in development. Kernel d represents a gene change that occurred early enough to affect the entire kernel.

The kernels in figure 6 illustrate the breaking up of orange to form a mosaic. The color segregation in kernel a, figure 6, is similar to those in figure 5. In kernel b, figure 6, there are two segments in which the orange color was separated into its component colors, red and white, while the space between them is typically mosaic. This mosaic segment apparently was produced by the same mechanism that produced the large segments but represents a number of distinct segregations that occurred later in development. In kernel c, figure 6, the segregation of orange into red and white extends over more than half of the pericarp, while in ear d, figure 6, the orange color was completely broken up into red and white.

The color changes in kernels b and c in figure 5, and a and b in figure 6 were not inherited, due no doubt to the fact that the gene change occurred in cells which took no part in the development of the embryo sac. Kernels like c, figure 6, which have a large segment of the surface changed to mosaic, grow into plants which produce ears with orange or mosaic pericarp, depending upon whether or not the change affected the embryo sac, providing, of course, they were fertilized by a colorless strain. In general, the larger the color change the more likely it is that the effect has extended to the sporogenous cells and the change is inherited.

DISCUSSION

Like all variegations, mosaic pericarp is inconstant in the relative amounts and distribution of the contrasting characters of the variegation. Although all variegations are highly inconstant, a study of a number of different variegations has shown that they are constant in their inconstancy. The color and pattern variations that appear in the mosaic

strain of maize are in all respects similar to those that occur in the calico type of variegation described by EMERSON (1913, 1917) and by EYSTER (1924), and to the color and pattern changes in a variegated strain of *Hesperis matronalis* as described by DE VRIES (1910). Some characteristics which are common to the three series of variegations mentioned above, as well as, probably, to all other variegations, are the following:

1. A variegation consists of contrasting characters segregated in the somatic tissue, and often also in the reproductive tissue.

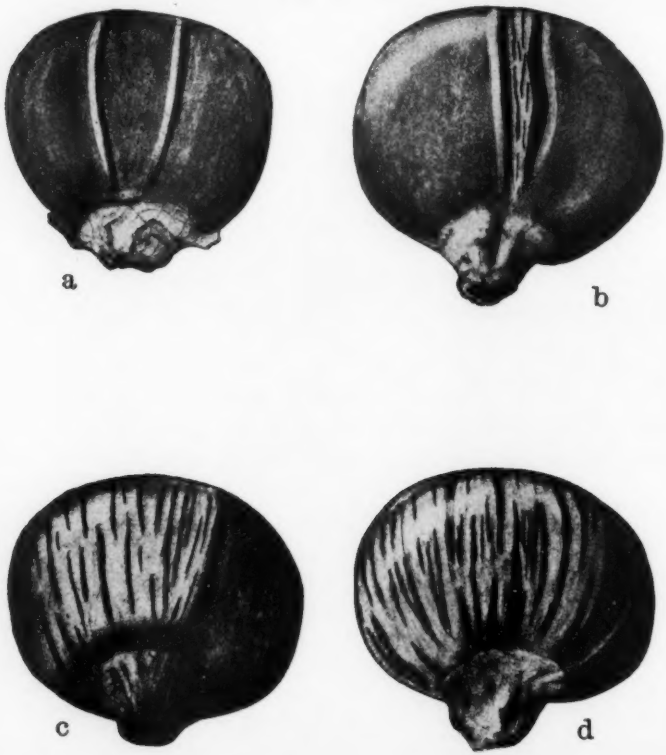


FIGURE 6.—Kernels which illustrate the breaking up of the orange color to form a mosaic: a represents a simple separation into red and white segments; b has two large color segregations and many smaller ones, so that part of the surface has the orange color broken up into a red-and-white mosaic; c has more than half of the orange surface broken up into red and white to form a typical mosaic; d has the orange completely broken up to form a mosaic. Drawings by GEORGE KLINE.

2. Variegations vary in pattern according to the relative amounts and distribution of the contrasting characters so as to form an intergrading series of patterns from one extreme to the other.

3. Color variegations give rise to an inconstant self-color which is intermediate between the contrasting characters of the variegation.

4. The intermediate self-color breaks up into the contrasting characters of which it is composed. The breaking up occurs in such a way as to make it probable that it is done by the mechanism of mitosis.

5. Variegations give rise to strains that breed true for one or the other of the contrasting characters. Such strains have lost the inconstancy peculiar to the variegated types.

If all variegations are fundamentally alike they should appear in a variety of patterns, give rise to an intermediate but inconstant type (like orange pericarp in maize), and produce constant types with only one or the other of the contrasting characters of the variegation (like the constant colorless and self-red strains that originate from the variegated strains in maize).

The color segregation in the pericarp of the orange kernels shown in figure 5 is such as to produce what are commonly known as sectorial chimeras. The replacement of the orange by equal and adjacent segments of red and white is good evidence that the mechanism of this color segregation is that of mitosis. So far as observations have been made, there appears to be little or no difference in color intensity in the homozygous orange strains as compared with orange strains heterozygous for colorless pericarp. Since one gene produces an orange pericarp not visibly different from that produced when two genes are present it is hardly likely that the somatic segregation of the orange to form adjacent segments of red and white is due to a non-disjunction of the chromosomes carrying the pericarp color gene. It is rather due to the non-disjunction of the contrasting particles within the gene.

The fact that the change from orange to mosaic is reversible and that this change involves the mechanism of mitosis would seem to indicate that the corresponding gene change is physical rather than chemical in nature. The gene for orange extends the pigment, though in reduced amount, throughout the pericarp tissue, while the gene for mosaic permits its contrasting elements to be segregated by mitosis. The relation of orange and mosaic is such as to suggest that their genes are fundamentally alike in content but have different structural arrangements so that they may be said to be isomeres. According to this view the change from orange to mosaic, or the reverse, would be the result of a structural

rearrangement of the same gene elements. The fluctuating variability in the intensity of orange color and in mosaic pattern might be due to small differences in gene content which would result from slight irregularities in division or chance distribution of the gene substance in mitoses.

SUMMARY

Mosaic pericarp is a coarse type of variegation which has the inconstancy typical of variegations. A large number of mosaic patterns, an inconstant dilute-red or orange type, constant colorless, and constant red types originated in pedigreed cultures from a single parent plant. These variations are inherited and form a series of multiple allelomorphs with the following order of dominance: self-red, heavy-mosaic pattern, light-mosaic pattern, orange and colorless. The frequencies of the different color and pattern changes have been determined.

The color and pattern variations in mosaic are similar to those found in other variegations. Deductions have been made as to the mechanism of variegations and the nature of the gene.

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